PR Microplate Reader

87360
87364

User Manual

Manual code : 87362
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Revision of the manual : October 1997
Software versions End Point 2.0 (SB)/1.3 (MB) Main Menu 2.02 (SB) / 1.33 (MB)
English

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DECLARATION OF CONFORMITY

- according to ISO/IEC Guide 22 and EN 45014

Manufacturer's Name:	**DYNEX TECHNOLOGIES**
Manufacturer's Address:	**DYNEX TECHNOLOGIES, Inc.** 14340 Sullyfield Circle Chantilly, VA 20151-1683 USA
declares that the product:	**Microtiter® Plate Reader**
Model:	MRX, MRX $_{II}$, MRX $_{PLUS}$, MRX $_{REVELATION}$
Product Options:	All

conforms to the following Product/Generic Specifications:

Safety:	EN 61010 - 1 : 1993 IEC 1010 - 1 : 1990 plus AMD 1 : 1992 BS EN 61010 - 1 : 1993
EMC:	EN 50081 - 1 : 1992 EN 50082 - 1 : 1992

Supplementary Information:

The product referred to above was tested in a typical configuration and complies with the requirements of Low Voltage Directive 73/23/EEC and EMC Directive 89/336/EEC.

Note: If changes are made to this product, including the use of unapproved material or peripheral equipment, then this declaration of conformity is invalid.

Chantilly, Virginia
USA
April 17, 1998

John L. Eccles
Director of Quality and Regulatory Affairs

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NOTICE TO OUR CUSTOMERS

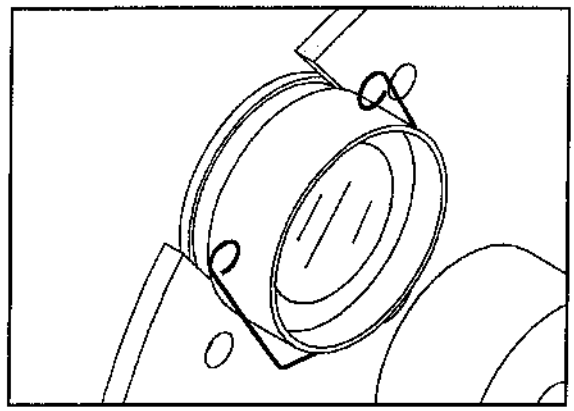
Addendum To PR Series Reader User Manual

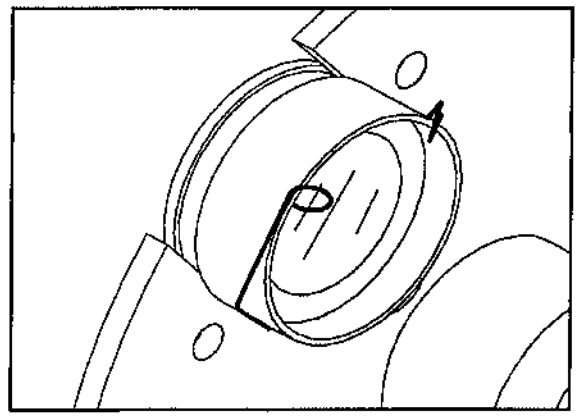
Affecting Chapter 2 "Installation and Setup",
under "Fitting the Lamp and Filters"

1. The Filter Blanks must be fitted into the unoccupied filter positions in the Filter Wheel.

2. Ensure the Filter Springs are around the sides of the Filter as shown in the top diagram. The Springs must not extend past the rear face of the Filter (bottom diagram). Otherwise, the Self-test will fail due to "Filter Motor Errors".

Correct Filter Spring Positioning
(View of Filter Wheel from filter spring side)





Incorrect Filter Spring Positioning

CERTIFICATE OF COMPLIANCE

DYNEX Technologies certifies that **PR2100** Serial No. **1CXD**

is YEAR 2000 compliant according to the following definition:

Definitions.

Definitions.

Year 2000 Compliant.

DYNEX Technologies has adopted the following definition of Year 2000 compliance as set forth by the British Standards Institute in DISC PD2000-1:
The definition of Year 2000 conformity Requirements (available on the Internet at http:/www.bsi.org.uk/disc/year2000/2000.html) has the qualification that the upper limit for compliance is the Year 2035.

Year 2000 conformity shall mean that neither performance nor functionality is affected by dates prior to, during, and after the Year 2000.

In particular

Rule 1. No value for current date will cause interruption in operation.

Rule 2. Date-based functionality must behave consistently for dates prior to, during, and after Year 2000.

Rule 3. In all interfaces and data storage, the century in any date must be specified either explicitly or by unambiguous algorithms or inferencing rules.

Rule 4. Year 2000 must be recognized as a leap year.

Signed

John L Eccles
Director of Quality & Regulatory Affairs

CONTENTS

1 Microplate Reader User Manual

2 Troubleshooting guide

3 Endpoint Program User Manual

PR Microplate Reader

User Manuals

Software Upgrades

When you receive software upgrades, the manuals will be supplied in shrink-wrapped form. These manuals are designed to replace the old manuals in your PR Manual Set Binder. If you wish to purchase another binder, please contact your Reader supplier.

To replace old manuals:

- Remove the old manual from the binder and discard it.
- Replace it with the new manual, taking care not to mix up pages.
- Remove the old troubleshooting guide from the binder and discard it.
- Replace it with the new troubleshooting guide, taking care not to mix up pages.

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Microplate Reader User Manual

Manual code : 87362

Revision of the manual : October 1997
Software versions Main Menu 2.02 (Single Board) / 1.33 (Multi Board)
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Limitations of Use

The user of the PR series Reader must follow the specific manufacturer's assay package insert when modifying parameters and establishing results calculation methods.

The performance characteristics of the PR series Reader have not been established with specific serology or antigen detection assays. The user must evaluate the PR series Reader in conjunction with the specific serology and/or antigen detection assays. This evaluation must include the establishment of performance characteristics for the specific assays.

Under no circumstances does the PR series Reader have any direct patient contact or perform any therapeutic patient function.

Specific diagnostic results are not provided by the device. Diagnostic decisions are made only after independent confirmation by additional methods under the supervision of a qualified professional.

WARNING:

If this equipment is used in a manner not specified by the manufacturer then the protection provided by the equipment may be impaired.

Before using any decontamination method, except those recommended by the manufacturer, users should check with the manufacturer that the proposed method will not damage the equipment.

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Table of Contents

About This Manual **1**

Safety Information **3**

Symbols **5**

Warning Symbol 5
Plate Carrier Diagram 5

Chapter 1 Features of the Reader **7**

Chapter 2 Installation and Setup **9**

2.1 Installation 11
Unpacking the Reader 11
The Reader Back Panel 12
The Reader Front Panel 14
The Keypad and Display 14
Fitting the Lamp and Optical Filters 15
Switching the Reader On 16
Switching between DOS Applications 17
2.2 Setup Procedures 18
Setup Menu 19
Filters Menu 19
Clock Menu 20
System Menu 21
Maintenance Menu 23
Comms Menu 24
Memory Menu 25
Param Menu 26

Chapter 3 Printer Interface **29**

Chapter 4 Maintenance **31**

4.1 Routine Maintenance Procedures 33
4.2 Cleaning 33
Autoclaving 34
External Painted and Plastic Surfaces 34
Filters 34
Decontamination 34
4.3 Repacking the Reader 34
4.4 Replacing the Lamp 35
4.5 Changing the Optical Filters 36

Appendix A: Self-Test Diagnostics 39

Self-Test Sample Printouts 40

Appendix B: Accessories 41

Appendix C: Specifications 43

Software Specifications 43
Hardware Specifications 43
Performance 43
405 to 850nm Wavelength Range 44
340 to 400 Wavelength Range (Option) 44
Interface 45
Electrical 45
Physical/Environmental Conditions 45

Index 47

About This Manual

This manual has been written for the laboratory technician and describes how to use the Microplate Reader.

With the information in this manual, you can:

- Install the Reader
- Configure the Reader, using the Setup menu, to suit your particular needs
- Connect the Reader to a printer
- Perform basic maintenance procedures.

This manual also describes all the features and specifications of the Reader hardware and software.

For information on how to use any software you have installed, such as Endpoint or Barcode software, refer to the individual user manuals.

For information on how to connect the Reader to an external computer, refer to the *Endpoint Program User Manual.*

For explanations of error messages and possible solutions, refer to the *Troubleshooting Guide.*

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Safety Information

If there is any doubt or concern about the safety of the instrument, contact an approved service center.

Warning: Electric Shock Hazard

Although this instrument is fully insulated and earthed (grounded), it is important for all users to be aware of the potential hazard of using liquids in close proximity to an electrical supply. If any liquids are spilled, disconnect the instrument from the mains electrical supply immediately and clean the liquid up. DO NOT reconnect the electrical supply until the instrument has been fully inspected by an approved service engineer.

Take care when using non-rigid, non-standard microplates as spillages are more likely to occur.

To switch this instrument off, press the power switch on the front panel. Remove the mains power cable from the socket on the rear panel.

Warning: Incorrect Operation

Operating this equipment in ways other than detailed in this manual may impair the protection provided by the instrument.

- DO NOT operate this equipment with the covers removed as potentially lethal voltages are contained within.
- DO NOT operate this equipment with the safety earth (ground) disconnected.
- DO NOT install unauthorized cards, spare components or accessories as this may impair the safety of the Reader and will invalidate its warranty.
- DO NOT place any objects, or stand within 150mm of the front of the Reader as this will obstruct the plate carrier.
- DO NOT overfill the microplate wells as this may lead to liquid contamination of the wells and impair the safety of the Reader.
- BE PREPARED for unexpected plate carrier movement when the Reader is controlled by an external computer.
- MAKE SURE that the voltage ratings on the rear panel of the Reader correspond to the local mains supply.

- MAKE SURE that the mains power cable is correctly wired. Color codes are as follows:

Europe		**United States**	
Brown	Live	Black	Live
Blue	Neutral	White	Neutral
Green/yellow	Earth (ground)	Green	Ground

Fuses

The instrument does not contain any user-replaceable internal fuses. However, the power supply contains an internal fuse in the primary circuit. If the fuse needs to be changed, it must be replaced with a CSA approved 20x5mm, 4A cartridge fuse with a voltage rating of 250V. This fuse **should not** be replaced by the user. If the fuse blows, contact an approved service center.

Symbols

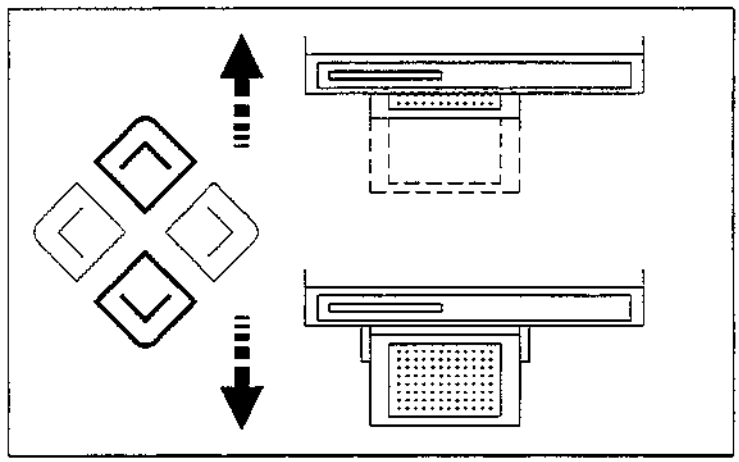
Warning Symbol

For your safety the Reader is marked with a warning symbol to remind you of certain hazards:

- On the back panel: this indicates that the optics door may be hot. If you want to remove the optics door, make sure that you allow the Reader to cool before touching it.
- Adjacent to the mains outlet: this indicates that the outlet should only be used for an external monitor with a power rating of up to 120VA and an earth leakage current of less than 1 mA at 264V and 60 Hz.

Plate Carrier Diagram

The diagram on the inside of the floppy disk drive door shows the mechanism for moving the plate carrier in and out.



While the Main menu is displayed, the plate carrier can be moved in and out using the up and down (∧ ∨) cursor keys. The up key moves the plate carrier in, and the down key moves it out.

Do not place any objects, or stand within 150mm (6.5 in) of the front of the Reader as this will obstruct the plate carrier.

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Chapter 1 Features of the Reader

The Microplate Reader is a microprocessor-controlled photometer. It is designed to measure the optical density (OD) of fluid samples in 96-well microplates in order to determine the level of chemical reactions.

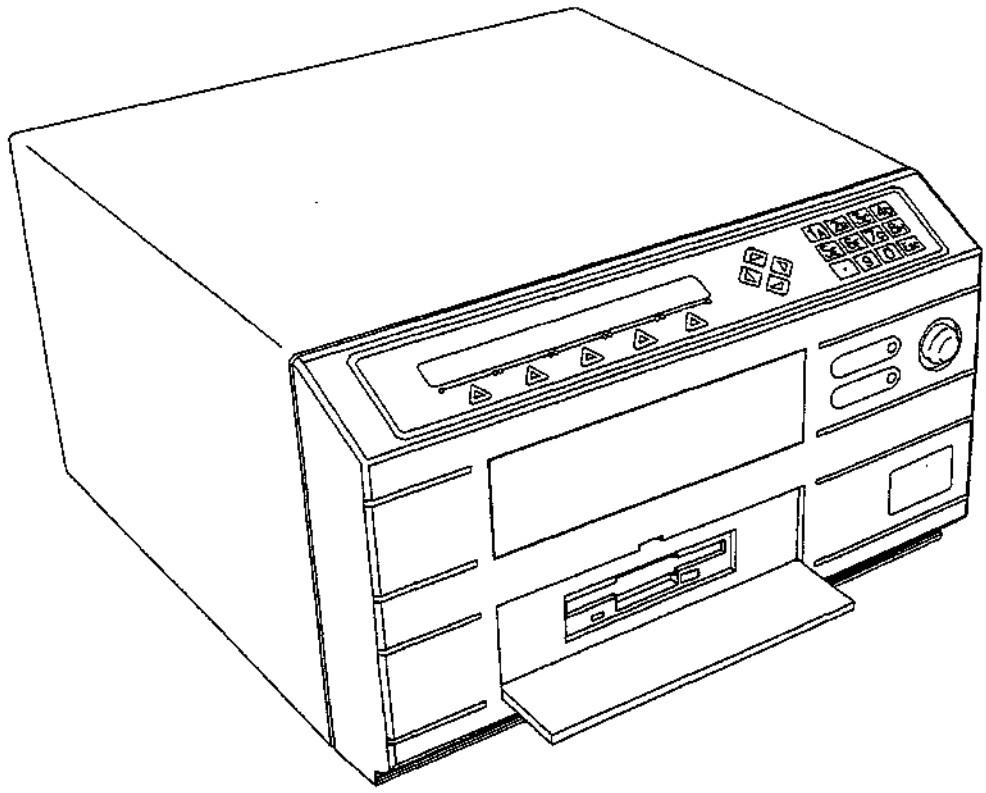

Figure 1 The Microplate Reader

A full list of the Reader's technical specifications can be found in Appendix C.

The Reader provides the following features:

- Endpoint Agglutination Software
- PC-compatibility for additional control and data storage
- Multi-lingual display which guides you through menu choices
- Single and dual wavelength reading modes
- Selection of up to six filter wavelengths
- Data analysis and presentation as OD matrices and/or graphically using curve fitting routines
- Data output to printer

- Storage of assay test procedures and microplate data on 3.5" floppy disk drive or hard disk
- Modular internal design making it quick to service and repair.
- Barcode option
- Test plate option
- Temperature Control option.

Chapter 2 Installation and Setup

2.1 Installation 11
Unpacking the Reader 11
The Reader Back Panel 12
The Reader Front Panel 14
The Keypad and Display 14
Fitting the Lamp and Optical Filters 15
Switching the Reader On 16
Switching between DOS Applications 17
2.2 Setup Procedures 18
Setup Menu 19
Filters Menu 19
Clock Menu 20
System Menu 21
Maintenance Menu 23
Comms Menu 24
Memory Menu 25
Param Menu 26

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2.1 Installation

CAUTION **DO NOT connect the instrument to the mains electrical supply or switch it on before you have completed this installation procedure.**

Unpacking the Reader

If you ordered an upgrade, you will only receive an upgrade disk and a manual.

The Reader is packed to provide maximum protection during shipment. Take care when unpacking it and examine it carefully for any damage. Report any damage to the carrier immediately. Check the contents against the shipping checklist enclosed and report any omissions to your supplier.

Remove the Reader from the carton and place it on a level surface free of dust, moisture, vibration, draughts and away from direct sunlight. (Keep the packing materials so that they can be used if the Reader has to be transported.)

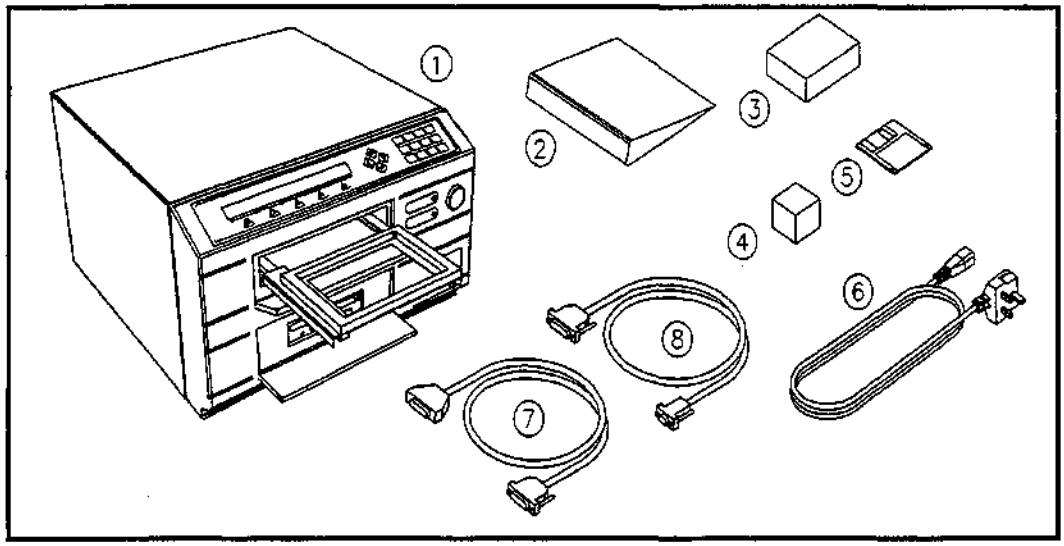

Figure 2 Example Packing Contents

1	Reader	5	Software disks
2	Manual Set	6	Power Cord
3	Optical Filters	7	Printer Cable
4	Lamp	8	Interface Cable
		9	RS 232 Filter (not shown)

The Reader Back Panel

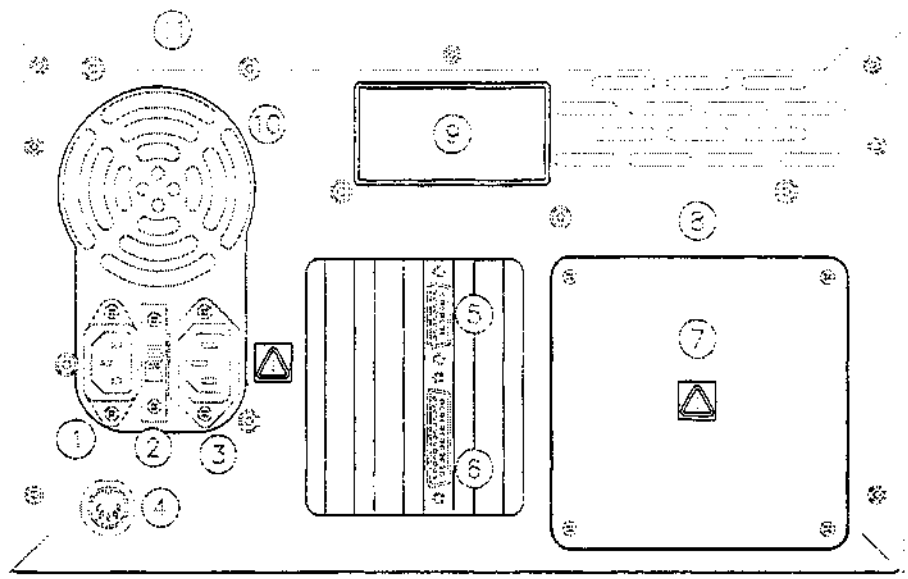

Figure 3 The Microplate Reader Back Panel

1	Mains Inlet	7	Optics Door
2	Voltage Selector (See the Voltage Selector section below)	8	Optics Cooling Vents
3	Monitor Mains Socket	9	Serial Number Plate
4	Keyboard Socket	10	PSU Cooling Vents
5	Serial Port	11	Main Enclosure
6	Printer Port		

Note: The warning symbols on the back panel indicate that the optics door may be hot, and that no appliances other than a monitor should be plugged into the mains outlet. For more details, refer to the discussion on Symbols earlier in this manual.

Note: The positions of ports 5 & 6 may not be exactly as shown, but the number of pins or pin sockets in each port will remain the same.

Note: Remove the port protectors, connector protector and styrofoam packing inside #7.

Voltage Selector

The position of the voltage selector (see Figure 3) is very important and must be adjusted to match the line voltage of the mains power supply **before** switching the Reader on.

Position of Voltage Selector	Mains Line Voltage
115V	100-120V
230V	200-240V

CAUTION **FAILURE TO FOLLOW THE PROCEDURES DETAILED BELOW MAY RESULT IN DAMAGE TO THE MICROPLATE READER DUE TO PARTS FALLING INTO THE POWER SUPPLY.**

There are **two** types of plastic cover fitted to the voltage selector switch:

- If the plastic cover has a slot cut in it and the voltage selector is not in the correct position, insert a suitable small flat-bladed screwdriver through the slot and move the selector to the desired voltage. DO NOT LOOSEN THE SCREWS USED TO RETAIN THE PLASTIC COVER.
- If the plastic cover does not have a slot cut in it and the voltage selector is not in the correct position, use a No. 1 Posi driver to remove **one** of the screws and loosen the other screw to allow the plastic cover to be pushed aside. Move the switch to the desired voltage, replace the cover and tighten the screws. DO NOT REMOVE BOTH SCREWS USED TO RETAIN THE PLASTIC COVER.

The Reader Front Panel

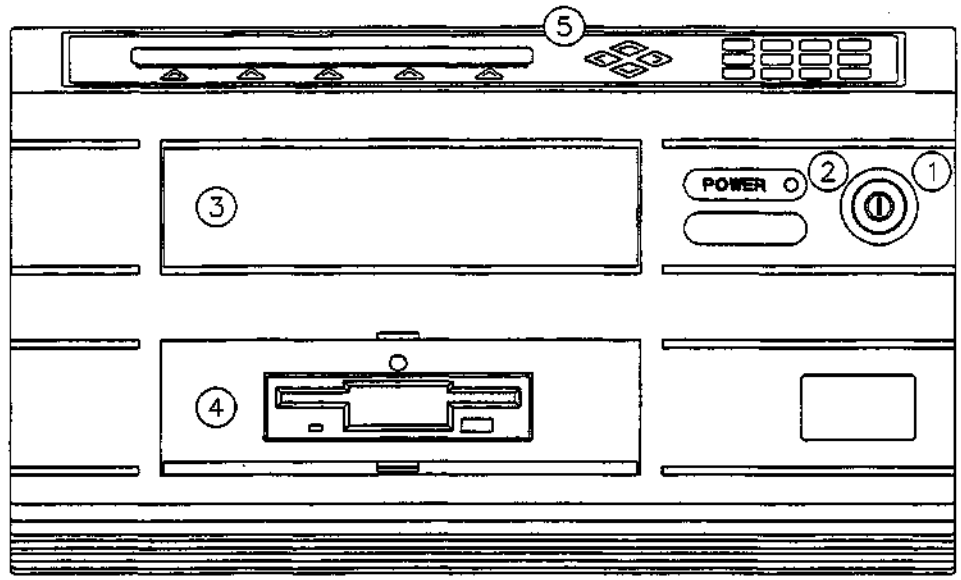

Figure 4 Microplate Reader Front Panel

1 Power Switch (marked **I/O** for on/off)
2 Power Light
3 Plate Carrier Door
4 Floppy Disk Drive
5 Keypad and display

The Keypad and Display

The Reader is controlled by menu-driven software which responds to the user's menu choices. The menus are displayed on a liquid crystal display which can show up to five options at a time. Choices are entered using the keypad.

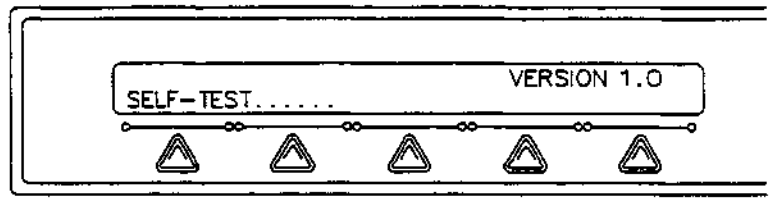

Figure 5 Function Keys and Display

- **Function keys** – menu options are shown on the bottom line of the display and selected by pressing the function key below.

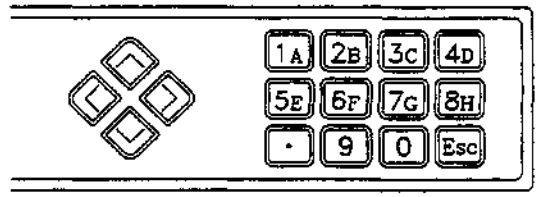

Figure 6 Alphanumeric Keys and Cursor Keys

- **Alphanumeric keys** – used to enter numbers and well locations.
- **Esc key** – returns the display to the Main menu.
- **Cursor keys** – used to scroll through well locations and menus, and (in some menus) to move the plate carrier in and out.

☞ *Note: When the Reader is operating under the control of an external computer, the keypad will not operate.*

Fitting the Lamp and Optical Filters

Warning **Disconnect the mains cable before fitting lamp & filters.**

☞ *Note: DO NOT touch the filters, bulb or reflector directly.*

1. Using a No. 1 Posi driver remove the optics door and, if fitted, remove the packing materials from behind the door.
2. Slide the optics assembly out of the instrument.

   If you have an older instrument (check your serial number panel: newer Readers have 1CXCxxxx or 2CXCxxxx numbers), you need to disconnect the optics assembly's 10-way connector and then slide the assembly out of the instrument.
3. Insert the lamp into the assembly and plug the connector into its back (see Figure 7).

   For Osram type lamps:
   Make sure that the lamp is seated correctly with the locating pip on the lamp fitted into the notch on the optics assembly.

   For Philips type lamps:
   Make sure that the lamp is seated correctly and that the lamp pins are horizontal as shown in Figure 7.
4. Insert the filters into the filter wheel.
   - Place the filter with the lowest wavelength in position 1, the next lowest in position 2 and so on.
   - Make sure the side of the filter that has the shorter distance from the groove to the end is toward the lamp.
   - Fit filter blanks into all unoccupied positions.
   - Ensure that the retaining springs grip the filter casing and are not bent forwards over the edge of the casing.
5. Slide the optics assembly back in *(if you have an older instrument, you must also reconnect the 10-way connector)* and replace the optics door. Attach the lamp connector to back of lamp.

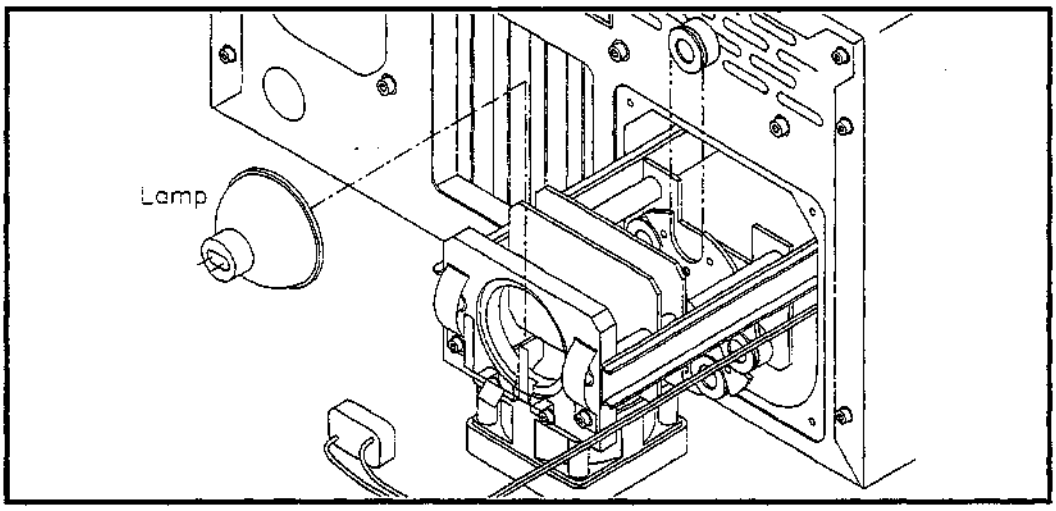

Figure 7 Fitting the Lamp and Filters

Switching the Reader On

Before switching the Reader on, note the position of the voltage selector (see Figure 3). The position of the selector is very important and must be adjusted to match the line voltage of the mains power supply.

Position of Voltage Selector	Mains Line Voltage
115V	100-120V
230V	200-240V

If the voltage selector is not in the correct position, refer to the voltage selector discussion in "The Reader Back Panel" earlier in this section.

DO NOT place any objects, or stand within 150mm of the front of the Reader as this will obstruct the plate carrier.

1. Make sure that the instrument is switched off. The power switch is ON when it is flush with the front panel and OFF when it stands out from the panel.
2. For PR1100 Readers: insert a Reader program disk into the floppy disk drive.
3. Insert the power cord supplied into the socket at the rear of the instrument.
4. Connect the power cord to a suitable power source with a protective earth (ground).
5. Connect the printer cable from the Reader to the printer.
6. Switch the printer on.
7. Switch the Reader on by pressing the power switch on the front panel.
8. Verify that the Reader completes the self-test routine successfully.

Self-Tests

When the Reader is switched on, it carries out several self-tests to check that the instrument is working correctly. Appendix A shows a list of these tests and a sample printout. During the self-tests, a series of symbols are displayed on the bottom line of the LCD: these indicate the optional extras that are installed in your Reader.

If the Reader fails any of the tests, an error message is displayed. If the Reader's data files have been corrupted, the Reader requests your serial number (this can be found on the Reader's back panel or inside of the floppy disk drive's door). If an error message persists, contact the service center.

Maintenance Reminder

On first power up, the Reader may display a maintenance reminder.

```
HAVE YOU PERFORMED YOUR
MAINTENANCE CHECKS ?
```

To clear the message, press any key. The reminder is intended for use by laboratories which routinely verify the operation of their instruments.

The self-test printouts and the frequency of the maintenance reminders may be altered from the **SETUP** menu.

Main Menu

If no faults are detected, the instrument displays the Main menu which shows all the available software programs and **SETUP**. If there are more than four software programs installed, use the < and > cursor keys to view them.

While the Main menu is displayed the plate carrier can be moved in using the ∧ cursor key and out using the ∨ cursor key. The plate carrier should not be left out of the Reader for more than a few minutes at a time as it will gather dust and may become damaged. If it is left for five minutes without using the software, the Reader will move the plate carrier in automatically.

Switching between DOS Applications

On a PR1100:

1. Press the Esc key to return the Reader to the Main menu.
2. Switch the Reader off.
3. Change the software disk.
4. Switch the Reader on again.

☞ *Note: If you have changed any settings in the Setup menu, you will have to re-enter them for the new program.*

On a PR2100:

1. Press the Esc key to return the Reader to the Main menu.
2. Select the required program using one of the function keys.

2.2 Setup Procedures

The Reader has a number of settings which can be adjusted to suit the assays which will be run on it. The settings are altered using the Setup menu. To use the Setup menu options, select **SETUP** from the Main menu. The options accessed by this menu are summarized in the table below and described in detail in this section.

Menu	Setting	Value
FILTERS (p. 19)	Number of Filters	1/2/3/4/5/6
	Wavelengths	340 – 850 nm
CLOCK (p. 20)	Time mode	12/24 hour
	Date mode	dd.mm.yy/mm.dd.yy
SYSTEM (p. 21)	Language	English/French/Italian German/Spanish
	Display	2x40
	Keypad	21 key
	Printer	various
MAINT. (p. 23)	Print self test	yes/no
	Retest	yes/no
	Reminder	no/daily/weekly/monthly
COMMS (p. 24)	Comms modes	MRX/MR700/Custom
	Comms Ports	COM1
PARAM (p. 26)	Over/Under limit	0 – 4.000 (3.500)
	Data Conversion	yes/no
	Plate Type	12x8/10x4
	Repeatability	1 – 4 readings

Note: If you are using a PR1100 Reader and you make changes to the Setup settings, these changes will only apply to the current software program. If you insert a different program disk, you will have to re-enter the settings.

Setup Menu

The Setup menu allows you to view and if necessary alter, the Reader settings.

```
SETUP ?          4:45P 30.06.94
 OTHER FILTERS CLOCK SYSTEM   CE
```

Select **OTHER** to access a second Setup menu

```
SETUP?           4:45P 30.06.94
 MAINT. COMMS MEMORY PARAM   CE
```

In the menus shown on the following pages, the options available are shown on the bottom line of the display. To select an option press the function key below it or, where appropriate, key in a value.

Note: In most of the menus the default or previously defined value is shown. To enter a different value you must press CE. This will access the alternatives available.

Example To change the display language from English to Spanish:
Select **SYSTEM** from the Setup menu to access the System menu. Select **LANGUAGE** from the System menu to access the Language menu.

The default or previously defined menu language is displayed:

```
LANGUAGE : ENGLISH   4:45P 30.06.94
                ENTER   CE
```

To access the alternative languages select **CE**.

```
LANGUAGE ?       4:45P 30.06.94
ENGLISH FRANCAIS DEUTSCH ITALIANO OTHER
```

Select one of the languages displayed by pressing the function key directly below it, or select **OTHER** to access the Spanish option.

```
LANGUAGE ?       4:45P 30.06.94
ESPANOL                CE
```

To select Spanish as the display language press the function key below **ESPANOL**. The menus will then be displayed in Spanish.

Filters Menu

The Filters menu allows you to install new filters and enter filter wavelengths.

Select **FILTERS** from the Setup menu to display the number of filters installed:

```
NO. OF FILTERS = 2   4:45P 30.06.94
                ENTER   CE
```

ENTER Confirm the number of filters shown.

CE Clear the number of filters and enter a new number. Minimum: 1 filter. Maximum: 6 filters.

Once the number of filters has been entered, the Reader prompts you to enter the wavelength of each filter in turn.

Note: When installing new filters, remember that the filter with the lowest wavelength should be in position 1 on the filter wheel, and so on in ascending order of wavelength.

The Reader will display Filter 1 and a wavelength, for example:

```
FILTER 1=410 nm      4:45P 30.06.94
                  ENTER  CE
```

Select **ENTER** to confirm the wavelength shown and move on to Filter 2. Select **CE** to clear the wavelength shown, and enter a new wavelength. Press **ENTER.**

When wavelengths have been entered for all the filters installed, the display will return to the Setup menu.

Clock Menu

Select **CLOCK** from the Setup menu to access the Clock menu and view the time and date settings.

```
SETUP?               4:45P 30.06.94
 TIME  DATE                  CE
```

Time

Select **TIME** to set the clock mode and time. The Reader will display the current setting. Select **ENTER** to confirm the mode shown, or select **CE** to display the alternatives.

```
CLOCK MODE ?         4:45P 30.06.94
12-HOUR 24-HOUR             CE
```

Select **12-HOUR** or **24-HOUR** mode.

The Reader will allow you to change the time display.

```
TIME =               4:45P 30.06.94
                  ENTER  CE
```

Select **ENTER** to confirm the time shown or enter the correct time using the keypad.

- If you selected the 24-hour mode, enter the time and press **ENTER.**
- If you selected the 12-hour mode, enter the time and then select **A.M.** or **P.M.**

Date

Select **DATE** to display the date mode. The Reader will display the current setting. Select **ENTER** twice to confirm the mode shown, or select **CE** to display the alternatives.

```
DATE MODE ?          4:45P  30.06.94
dd.mm.yy      mm.dd.yy          CE
```

Select **dd.mm.yy** or **mm.dd.yy**.

The Reader will allow you to change the date display.

```
DATE =               4:45P  30.06.94
                   ENTER   CE
```

Select **ENTER** to confirm the date shown or type in a new date. To edit the date entered, select **CE**, use the keypad to enter a new date, and then select **ENTER**.

System Menu

Select **SYSTEM** from the Setup menu to access the System menu and view the printer and user interface settings.

```
SETUP ?              4:45P  30.06.94
LANGUAGE DISPLAY KEYPAD  PRINTER   CE
```

Language

Select **LANGUAGE** to set the language options. The Reader will display the current language setting. Select **ENTER** to confirm this setting or **CE** to access the options:

```
LANGUAGE ?           4:45P  30.06.94
ENGLISH FRANCAIS DEUTSCH ITALIANO OTHER
```

OTHER accesses a second Language menu with the **ESPANOL** option.

Select the required language, or select **CE** to return to the System menu. The language chosen will be used to display all subsequent menus.

Display

Select **DISPLAY** to view the Reader display format.

```
DISPLAY : 2 X 40     4:45P  30.06.94
                   ENTER   CE
```

Press **CE** or **ENTER** to return to the System menu.

Keypad

Select **KEYPAD** to view the Reader keypad format.

```
KEYPAD : 21 KEY      4:45P 30.06.94
                  ENTER  CE
```

Select **ENTER** to move on. The keypad has an optional beep which sounds each time a key is pressed. The Reader will display the current key beep setting. Select **ENTER** to confirm the setting, or select **CE** to access the options:

```
KEY BEEP?          4:45P 30.06.94
 YES  NO                 CE
```

Select **YES** to switch the beep on, or **NO** to switch it off.

Printer

Select **PRINTER** to view the printer settings.

```
EPSON LX-810       4:45P 30.06.94
 NEXT  LAST       ENTER  CE
```

Select **ENTER** to accept the printer shown or use the **NEXT** and **LAST** commands to scroll through the list of printers.

The Reader is supplied with a number of standard printer drivers. If your printer is not listed here, contact the service center.

Page Size

The Reader asks you to enter the page size (in inches). For example:

```
PAGE LENGTH = 11.000   4:45P 30.06.94
                  ENTER  CE
```

Select **ENTER** to accept the page length and width shown or press **CE** to enter new values.

Lab Name

The Reader asks if you want to print a laboratory name at the top of each print-out sheet.

```
LAB. =             4:45P 30.06.94
   YZ ABCD           ENTER  CE
```

Enter a laboratory name, or select **ENTER** to return to the System menu.

Laboratory names may be up to 16 characters in length and consist of the following characters.

- Numbers 0–9 and a decimal point.
  Enter these using the keypad.
- Upper-case letters, lower-case letters and symbols.

Enter these in the following way:

a) Choose upper-, or lower-case letters or the symbols using the ∧ and ∨ cursor keys.

b) Scroll through the display characters on the display using the < and > cursor keys.

c) Enter the character shown by the flashing cursor by pressing the ∧ function key below it.

When the name is complete, select **ENTER**.

Maintenance Menu

Select **MAINT.** from the Setup menu to access the Maintenance menu and view the self-test printouts and maintenance reminder settings.

Self-Tests

Self-tests are performed when the Reader is switched on, to make sure everything is working correctly. The self-test can be printed, if necessary, so that a record can be kept. The Reader will display the current self-test print setting. Select **ENTER** to confirm the setting or **CE** to access the options.

```
PRINT SELF-TEST ?     4:45P 30.06.94
 YES   NO                  CE
```

Select **YES** to print the self-tests each time they are run.

☞ *Note: The printer must be connected directly to the Reader for the self-test to print.*

An extra self-test (retest) is also available which is carried out before each plate is read. The Reader will display the current setting for the test. Select **ENTER** to confirm the current setting or **CE** to access the options.

```
TEST BEFORE READ ?    4:45P 30.06.94
 YES   NO                  CE
```

Select **YES** to perform the extra test or **NO** to omit it.

☞ *Note: Adding the extra test will lengthen the reading time.*

Maintenance Reminder

The Reader has a maintenance reminder which can be displayed at regular intervals on power up. The reminder is intended for use by laboratories which routinely verify the operation of their instruments.

The Reader will display the current setting. Select **ENTER** to confirm the current setting or **CE** to access the options.

```
MAINTENANCE ?        4:45P 30.06.94
 NO  DAILY  WEEKLY  MONTHLY   CE
```

Select **NO** if you do not want any reminders to be displayed, or select the appropriate frequency.

Comms Menu

Select **COMMS** from the Setup menu to access the Communications menu and view PC communications settings.

Note: Do not alter the default settings unless you are proficient in RS232 communications protocols.

```
SETUP ?              4:45P 30.06.94
 MR700   MRX   USER          CE
```

MR700

Select **MR700** to set the Reader communication parameters to a format suitable for all Dynex application programs. The MR700 settings are:

Baud Rate	9600
Stop Bits	1
Data Bits	8
Parity	even
Data Format	MR700

Communications Port

The Reader asks which communication port is being used. Select **COM1** or **COM2** to specify the port, or select **CE** to return to the Comms menu.

MRX

Select **MRX** to set the Reader communication parameters to an enhanced format suitable for most application programs. When MRX is selected, the display flashes briefly and then redisplays the setup menu.

The MRX settings are:

Baud Rate	9600
Stop Bits	1
Data Bits	8
Parity	even
Data Format	MRX

Communications Ports

The Reader asks which communication port is being used. Select **COM1** or **COM2** to specify the port, or select **CE** to return to the Comms menu.

Note: When using a PR1100 or a multiboard PR2100, no COM2 option exists.

User

Select **USER** to customize the communication settings. The User options allow you to set the values for baud rate, stop bits, data bits, parity and data format.

The settings available are:

Baud Rate	110/150/300/600/1200/2400/4800/9600
Stop Bits	1/2
Data Bits	7/8
Parity	none/even/odd
Data Format	MR700/extended/MRX

Memory Menu

Select **MEMORY** from the Setup menu to access the Memory menu which allows you to reset the Reader memory or to upgrade the software.

```
SETUP MEMORY ?      4:45P 30.06.94
 RESET  UPGRADE            CE
```

To upgrade the Reader, follow the instructions provided with the upgrade disk.

Reset

The **RESET** command resets the Reader's default settings, overwriting all user-defined setup parameters. The Reader will ask you to confirm this action.

```
ARE YOU SURE ?      4:45P 30.06.94
 YES   NO                 CE
```

Select **YES** to reset all the Reader's parameters, or **NO** to return to the Memory menu.

Upgrade

The **UPGRADE** option allows you to upgrade the Reader with a software upgrade disk. Only Reader application software obtained from your supplier should be used with this command.

If you are upgrading the Main menu Setup software, refer to "Upgrading From a Previous Version" in Chapter 3 in the *Endpoint Program User Manual.*

Param Menu

Select **PARAM** from the Setup menu to access the Parameters menu and view the OD limit, data conversion, plate type and repeatability settings.

Over Limit

The Over limit is used to set a range within which ODs should fall. ODs above the limit will not be processed. The limit is also used as an Under limit to set the negative limit of the range.

For example: If 3.500 is set as the over limit, ODs above 3.5 will not be stored and will be printed as "OVER". ODs below -3.5 will be printed as "UNDER".

The Reader will display the current Over Limit. Select **ENTER** to confirm the value shown and move on to the Param Plate Type menu. Select **CE** to clear the value shown, and enter a new value.
Minimum: 0.000. Maximum: 4.000.

Note: Take care when setting the Over limit; if it is too low valid test results may be rejected. The normal limit is 3.500.

Data Conversion

If the Over limit is altered, the Reader asks if ODs which lie outside the range should be assigned a conversion value for use in quality control equations. If no value is assigned to these ODs, the QC equations which contain them will substitute five asterisks (*****) for a well which is marked as OVER.

```
DATA CONVERSION        4:45P 30.06.94
 YES   NO              ENTER  CE
```

Select **YES** to enter a value for Over and Under ODs or select **NO** to define the plate type. The Reader will ask for the Over value, followed by the Under value. For example:

```
OVER VALUE =9.999              4:45P 30.06.94
                    ENTER  CE
```

Enter Over and Under values and select **ENTER**.

Min. Over value: 0.000. Max. Over value: 9.999.
Min. Under value: -9.999. Max. Under value: 0.000.

Plate Type

Two types of plate format can be used with the Reader. One is the standard 12 rows by 8 columns format and the other is the 10 rows by 4 columns format or the "Chinese plate" format.

The Reader will display the current plate type setting.

Select **ENTER** to confirm the current plate type setting, or **CE** to access the options.

```
PLATE TYPE ?          4:45P 30.06.94
 12x8   10x4                CE
```

Select **12x8** or **10x4** to the specify the plate type.

Repeatability Mode

Using the repeatability mode you can specify that each reading the Reader takes is repeated a number of times and averaged into a single result.

The Reader will display the current repeatability setting. Select **ENTER** to accept the current setting and return to the Setup menu, or select **CE** to enter a different number.

```
REPEATABILITY MODE =  4:45P 30.06.94
                    ENTER   CE
```

Minimum: 1 (no reading repeats). Maximum: 4.

Note: Repeated readings will require a longer reading time and this option should be used with caution for time-critical plate readings.

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Chapter 3 Printer Interface

If the Reader has been supplied with a printer, this printer will be completely compatible with it. The following information is for users whose printer was not supplied with the Reader.

Any printer which communicates through a Centronics parallel printer interface will operate successfully with the Reader.

☞ *Note: Once the printer has been correctly installed, it should always be switched on before switching on the Reader.*

An external printer can be connected to the Reader using the interface cable shipped with the instrument. This is a ribbon cable terminated by a standard IEEE / Centronics style, 36 way connector (see Figure 8).

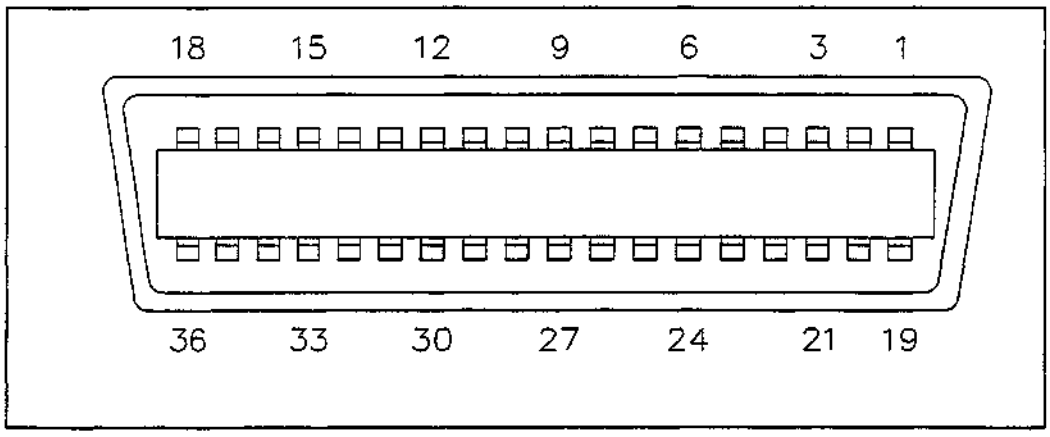

Figure 8 Printer cable connector (Centronics)

The table below shows the relevant pin assignments.

Pin No	Signal Name	Pin No	Signal Name
1	STROBE	19	GROUND
2	DATA 1	20	GROUND
3	DATA 2	21	GROUND
4	DATA 3	22	GROUND
5	DATA 4	23	GROUND
6	DATA 5	24	GROUND
7	DATA 6	25	GROUND
8	DATA 7	26	GROUND
9	DATA 8	27	GROUND
10	ACKNLG	28	GROUND
11	BUSY	29	GROUND
12	PE	30	GROUND
13	SLCT	31	INIT
14	Not Used	32	Not Used
15	Not Used	33	Not Used
16	Not Used	34	Not Used
17	Not Used	35	Not Used
18	Not Used	36	Not Used

Chapter 4 Maintenance

4.1 Routine Maintenance Procedures 33
4.2 Cleaning 33
Autoclaving 34
External Painted and Plastic Surfaces 34
Filters 34
Decontamination 34
4.3 Repacking the Reader 34
4.4 Replacing the Lamp 35
4.5 Changing the Optical Filters 36

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IMPORTANT

The first section in this manual contains safety information for all the equipment in the system. You should be familiar with this information anyway, but if not, you must read through this section before carrying out any maintenance.

The warranty on the Reader may become void if you do not follow the safety and maintenance instructions contained in this manual, or if the covers are removed by anyone other than approved service personnel.

4.1 Routine Maintenance Procedures

The Reader requires very little maintenance, however, certain simple procedures should be carried out on a regular basis.

On a daily basis:

- Verify self test passes.
- Remove the microplate from the plate carrier, wipe the plate carrier down and return it to the In position.
- Wipe down the external surfaces. Section 4.2 contains more detailed cleaning instructions.

On a weekly basis:

- Review the results of the self-test that occurs when you switch the Reader on to make sure the instrument is functioning correctly. If you want to retain this information for your records, print out the self-test results (see Appendix A for a sample) or save them to a file.
- Back up the data files for PR1100 (remove the disk and back it up using the File Manager program on an external PC).

On a yearly basis:

- Remove the filters and clean them. Section 4.2 contains more detailed cleaning instructions.

Periodic maintenance should be determined by the user.

4.2 Cleaning

Warning **Always switch the instruments off and disconnect the power cable before cleaning the Reader.**

The Reader is constructed from high quality materials, nevertheless spilt saline solutions, solvents, acids or alkaline solutions must be removed from outer surfaces immediately to prevent damage.

Autoclaving

DO NOT autoclave any part of this instrument.

External Painted and Plastic Surfaces

Clean external surfaces with a mild laboratory detergent, followed by 10% bleach or 70% alcohol. Always dilute detergents according to the manufacturer's instructions.

Filters

Clean the filters by wiping them with a lint-free cloth or a lens tissue. If necessary a little alcohol may be used with the cloth or tissue.

Decontamination

If you need to return the Reader to the service center, and it has been in contact with human blood, other potentially infectious body fluids, pathological samples, toxic or radioactive materials, it must be decontaminated.

Clean the surface with a mild laboratory detergent followed by 10% bleach before shipping the instrument.

4.3 Repacking the Reader

This procedure assumes that the original Reader packaging materials are being used. Figure 9 summarizes the repacking instructions.

1. Remove the optics door and take out the lamp and filters. When you replace the optics door, insert the optics door packing materials.
2. Pack the lamp and each of the filters separately.
3. Make that there is no microplate in the plate carrier, and no disk in the floppy drive.
4. Place the Reader in the original anti-static plastic bag.
5. Place the bottom end caps into the carton and lower the Reader into the carton. Fit the top end caps to the Reader.
6. Place the filters and lamp in the accessories box and fit this between the end caps.
7. Seal the box carefully and securely. Make sure that the box is not dropped or handled in a rough manner.

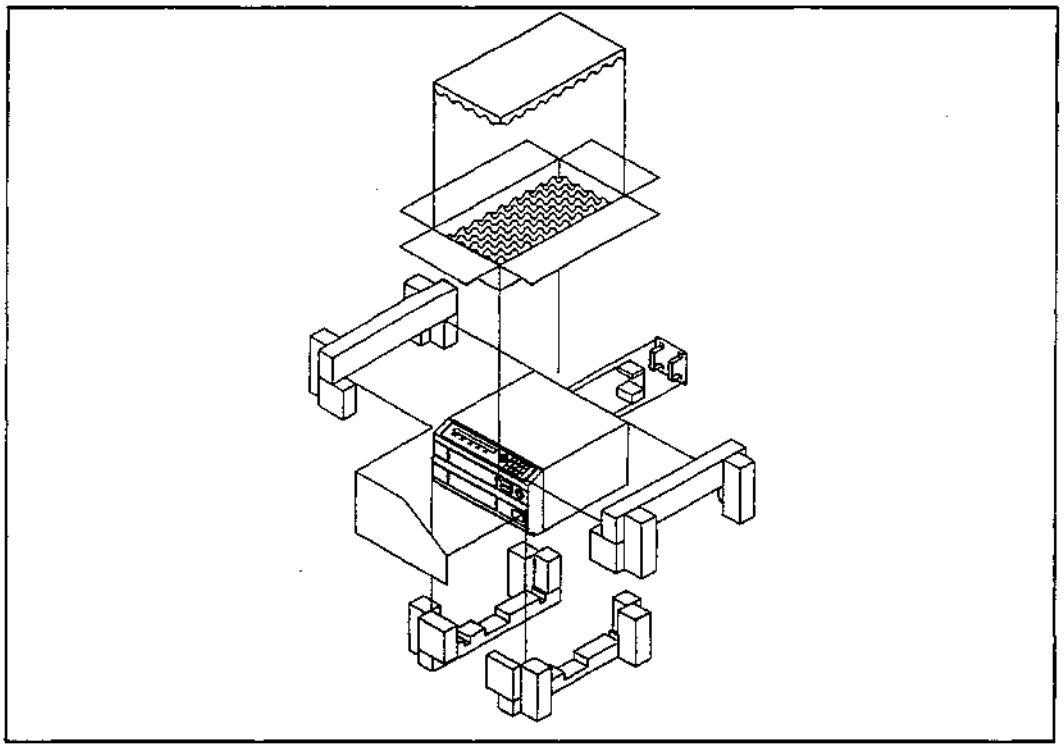

Figure 9 Repacking the Reader

4.4 Replacing the Lamp

Warning **If the Reader has been used recently, the optics door and the lamp in the optics assembly may still be hot. Allow time for the Reader to cool before touching it. Take care when removing the optics door as there may be broken glass in the optics assembly.**

Figure 10 summarizes the lamp replacement instructions.

☞ *Note: DO NOT touch the bulb or reflector directly.*

1. Switch off the mains power supply and disconnect the cable.
2. Using a No. 1 Posi driver remove the optics door.
3. Slide the optics assembly out of the instrument.

   If you have an older instrument (check your serial number panel: newer Readers have 1CXCxxxx or 2CXCxxxx numbers), you need to disconnect the optics assembly's 10-way connector and then slide the assembly out of the instrument.
4. Detach the lamp from the assembly and replace with a new one. Make sure that the lamp is seated correctly (refer to "Fitting the Lamp and Optical Filters" in Section 2.1).
5. Slide the optics assembly back in *(if you have an older instrument, you must also reconnect the 10-way connector).*

6. Replace the optics door and reconnect the power supply.

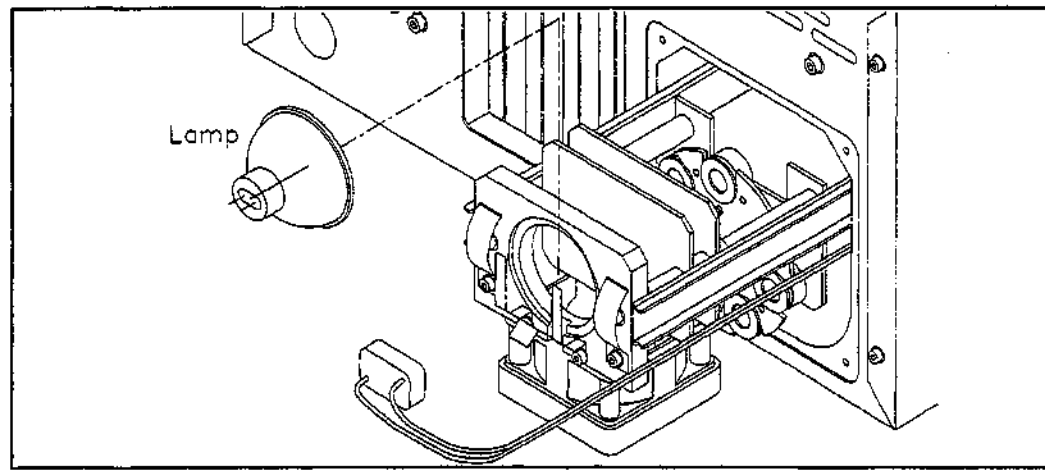

Figure 10 Replacing the Lamp

4.5 Changing the Optical Filters

Warning **If the Reader has been used recently, the optics door and the lamp in the optics assembly may still be hot. Allow time for the Reader to cool before touching it. Take care when removing the optics door as there may be broken glass in the optics assembly.**

Figure 11 summarizes these instructions.

☞ *Note: DO NOT touch the filters, bulb or reflector directly*

1. Switch off the power supply and disconnect the mains cable.
2. Using a No. 1 Posi driver remove the optics door.
3. Slide the optics assembly out of the instrument.

   If you have an older instrument (check your serial number panel: newer Readers have 1CXCxxxx or 2CXCxxxx numbers), you need to disconnect the optics assembly's 10-way connector and then slide the assembly out of the instrument.
4. Remove the relevant filter from its socket in the filter wheel, and replace with the new one.
   - Make sure the side of the filter that has the shorter distance from the groove to the end is toward the lamp.
   - Make sure the retaining springs grip the casing of the filter and are not bent forwards over the edge of the casing.
5. Slide the optics assembly back in *(if you have an older instrument, you must also reconnect the 10-way connector).*
6. Replace the optics door and reconnect the power supply.

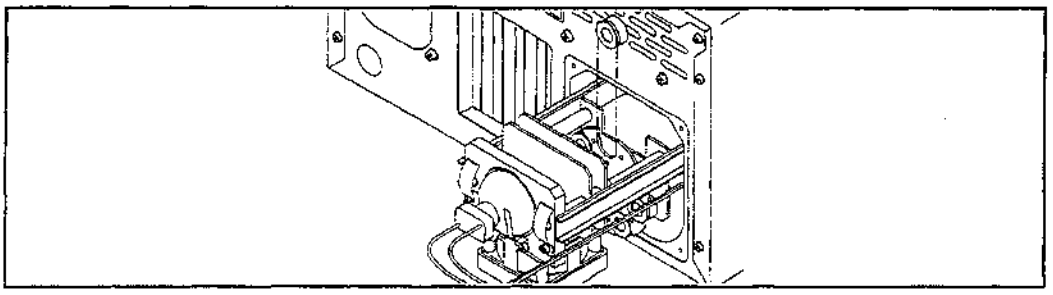

Figure 11 Changing the Optical Filters

☞ *Note: If you install new filters, you should run the test plate software. Be certain to run PARAMS to establish new baselines*

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Appendix A: Self-Test Diagnostics

On power up and (optionally) before every read the Reader performs several self-tests. These cover:

A/D Status	• Begins conversion and checks that the status line goes high and low. • Verifies that hardware can be detected and chosen.
Gain Selection	• Selects gain and compares with check-back circuit results. • Additional hardware check.
Photodiode Selection	• Selects all photodiodes and compares with check-back circuit results. • Additional hardware check.
Plate Motor	• Moves the plate to Out position and then to the In position. • Verifies that the plate carrier position sensors are operating correctly. • Checks motor and sensors.
Background Light	• Checks for light leakage into the reading compartment.
Bulb	• Verifies that current is flowing through the bulb when it is switched on. • Checks that the bulb has not blown.
Filter Motor	• Moves the filter motor to the first position. • Checks the filter motor and optical alignment.
Transmission Levels	• Verifies that all fibres are capable of transmitting at least 33% of the maximum transmission level. • Checks for blocked channels or broken diodes.
Filters	• Checks that gains can be selected for all filters. • Checks for damaged or missing filters.
Temperature Control	• Checks to see of the temperature control hardware is present. • Checks that communication can be established with the controller software.

Self-Test Sample Printouts

```
                    SELF-DIAGNOSTIC SEQUENCE

Reader Serial # A340                          Technologist: . .
. ..

Date              : 14.06.94
Time              : 08:53



Test 1            : A/D                       Pass
Test 2            : Gain selection            Pass
Test 3            : Photodiode                Pass
                  selection
Test 4            : Plate motor               Pass
Test 5            : Background light          Pass
                  levels
Test 6            : Bulb                      Pass
Test 7            : Filter motor              Pass
Test 8            : Transmission levels       Pass
Test 9            : Filters                   Pass


Self-Diagnostic Summary

Reader Status : READY
```

Appendix B: Accessories

Description	SDP Catalog No.	Dynex Part No. (for reference)
PR1100	87364	00111030
PR2100 (single board)	87360	00121010
Barcode Reader, Wedge	87170	0119780410
Cable, Printer	85637	1119760008
Cable, RS-232	87105	1119760007
Euro Power Cord	85622	6090405008
Filter 340nm	87106	6329780340
Filter 405nm	87369	6329780405
Filter 450nm	87366	6329780450
Filter 490nm	87368	6329780490
Filter 540nm	87371	6329780540
Filter 550nm	87118	6329780550
Filter 620nm	87367	6329780620
Filter 630nm	87119	6329780630
Filters 3 Pack (450/490/620)	87109	13000600
Filters 6 Pack (405/450/490/550/620/630)	87114	13000610
Lamp (UV)--shipped standard with single board unit	87128	6360408002
PR Dust Cover	87380	9119780012
PR Lens Cleaning Cloth	87108	41000070
PR Quick Servicing Guide (Service Manual single board)	87121	99000500
RS-232 Filter, 9 way D Connector	87122	50400380
SDP Endpoint Software for PR2100	87379	04000261
SDP Endpoint Software for PR1100		04000391
Test Plate 12 way (non UV) 450nm with Manual, Insert, and Software 2.0	87127	00140010
Test Plate Software version 2.0 (for use with single board unit only)	87124	04000400 04000410

US Power Cord	87129	6090405009
User Manual version 2.0	87125	99001720

Appendix C: Specifications

Software Specifications

Menu language	English/French/German/Italian/Spanish
Clock function	Day, date and time
MS DOS™ Software	Endpoint program option Agglutination program option
Program Load Time	Using PR1100: <40 seconds Using PR2100: <1 second

Hardware Specifications

Keypad	21 key tactile membrane
Display	2 line by 40 character backlit LCD
No. of filters	2-6

Performance

Read cycle time	*Single Wavelength:*	< 4 seconds
	Dual Wavelength:	< 6 seconds

405 to 850nm Wavelength Range

Dynamic range		-0.100 to +4.000 OD
Linearity†	*0.000 to 2.000 OD:*	± 1.0%
	2.001 to 3.500 OD:	± 1.5%
Filter band width	*(FWHM)*	10 nm ± 2 nm
Center Wavelength Accuracy		± 2 nm
Precision*	*0.000 to 2.000 OD:*	0.2% CV or 0.005 OD whichever is greater
	2.001 to 3.000 OD:	0.6% CV
	3.001 to 3.500 OD:	1.0% CV
Accuracy	*0.000 to 3.500 OD:*	2.5% or 0.005 OD whichever is greater

340 to 400 Wavelength Range (Option)

Dynamic range		-0.100 to +3.000 OD
Linearity†	*0.000 to 2.500 OD:*	± 2.0%
Filter band width	*(FWHM)*	18nm ± 2 nm
Center Wavelength Accuracy		± 2 nm
Precision*	*0.000 to 2.000 OD:*	0.6% CV or 0.005 OD whichever is greater
	2.001 to 2.500 OD:	1.0% CV

† Linearity is the maximum percentage deviation of a reading from the ideal straight line plot of OD versus concentration. (Measured by the manufacturer at 410nm in the visible range and 340nm in the UV range).

* Precision is the mean Coefficient of Variation (CV) figure for all wells on a plate that has been read 20 times in succession. (Measured by the manufacturer at 410nm in the visible range and 340nm in the UV range).

Interface

Printer Interface	Parallel Centronics Compatible with up to 850 printers. All printers must be compliant with EMC directive 89/336. A number of standard printer drivers are included. If your printer is not one of these, contact the service center.
Computer Interface	Bi-directional RS232C with user-definable protocol.

Electrical

Light source	75W tungsten halogen lamp
Mains input	100 to 120V or 200 to 240V 50 to 60 Hz
Power consumption	500 VA
Fuses	There are no internal user-replaceable fuses. The power supply contains a CSA approved 20x5mm, 4A cartridge fuse with a voltage rating of 250V in the primary circuit. If the fuse blows, contact the service center.
Mains Power Fuse (U.K. mains plug/powercord)	5A
Safety	Class I apparatus

Physical/Environmental Conditions

Weight	12.5 kg (27.5lb)
Dimensions	D390 x W360 x H220 mm
Operating Temperature	10 – 40°C
Operating Humidity	10 – 90% RH
Storage Temperature	0 – 50°C
Storage Humidity	10 – 90% RH
Altitude	< 2000m

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Index

—A—

Accessories, 41
Alphanumeric Keys, 14

—B—

Back Panel, 12

—C—

Cleaning, 33–34
Clock Menu, 20–21
Comms Menu, 24–25
Cursor Keys, 14

—D—

Decontamination, 34
Display, 14

—E—

Electric Shock Hazard, 3
External Painted and Plastic Surfaces
  cleaning the, 34

—F—

Filters
  cleaning the, 34
Filters Menu, 19–20
Front Panel, 14
Function keys, 14
Fuses, 4

—H—

Hardware Specifications, 43

—I—

Incorrect Operation, 3

—K—

Keypad, 14

—L—

Lamp
  fitting the, 15–16
  replacing the, 35–36

—M—

Main Menu, 17
Maintenance, 33–34
Maintenance Menu, 23–24
Maintenance Reminder, 17, 23
Memory Menu, 25
Microplate Reader. *See* Reader.

—O—

Optical Filters
  changing the, 36–37
  fitting the, 15–16
Over Limit, 26

—P—

Param Menu, 26–27
Performance specifications, 43–44
Plate Carrier Diagram, 5
Plate Type, 26
Printer interface, 29–30

—R—

Reader
- accessories, 41
- back panel, 12
- connecting a printer to it, 29
- electrical specifications, 45
- features of, 7–8
- front panel, 14
- hardware specifications, 43
- illustration of, 7
- interface, 45
- keypad and display, 14
- performance specifications, 43–44
- physical/environmental conditions, 45
- repacking the, 34–35
- resetting its memory, 25
- self-tests, 17
- software specifications, 43
- switching it on, 16
- unpacking the, 11

Repacking the Reader, 34–35
Repeatability Mode, 27
Resetting the Reader memory, 25

—S—

Safety Information, 3–4
Self-tests, 17, 23, 39
- sample printout, 40

Setup menu, 18–19
Software Specifications, 43
Specifications, 43–45
System Menu, 21–23

—V—

Voltage Selector, 13

—W—

Warning Symbol, 5

PR Microplate Reader

87360
87364

Troubleshooting Guide

Manual code : 87362

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English

sanofi
PASTEUR

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Table of Contents

About This Manual 1

Section 1 Error Messages 3

Section 2 Solving Problems 15

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About This Manual

This manual contains two sections:

- An **error messages section** that provides an alphabetic list of the error messages with an explanation of what they mean, and possible solutions.
- A **problem solving section** that provides a list of hardware, printing and general problems which may occur but are easily rectified

WARNING: ELECTRIC SHOCK HAZARD

The Reader contains no user-serviceable parts.

The instrument must not be operated with the covers removed – potentially lethal voltages are contained within.

If any fault occurs with the Reader for which you cannot find an explanation in this manual, contact the nearest approved service center immediately.

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Section 1 Error Messages

The Reader carries out a series of self-tests immediately after it is powered-up. Failure of some of these tests does not necessarily stop the Reader from working. Two types of failure can occur: fatal and non-fatal errors.

- **Self-Test Fatal Errors** – some fatal errors may prevent the Reader from working completely. Others will display a warning message but allow the self-tests to continue. If the Reader displays a fatal error message, switch the Reader off and disconnect it from the main power supply. Contact the nearest approved service center for help and advice.
- **Self-Test Non-fatal Errors** – these will allow the Reader to carry on operating but the Reader's measurements will be subject to certain restrictions.

The Reader generates a number of messages during normal operation. However, if a fault occurs, the Reader may display an error message. These messages are designed to help you locate the cause of the problem, and they often suggest possible solutions. If the problem persists, contact an approved service center.

Error Message	Problem/Recommended Action
A/D Status error	*Contact an approved service center*
Agglutination assays cannot be run together unless they have the same speed...	You cannot combine agglutination assays onto the same plate, unless they have the same wavelengths, readings per well and plate speed settings. *Change the assays so that they can be run together.*
Agglutination assays cannot be run with other types of assay	You cannot combine agglutination assays onto the same plate with non-agglutination assays. *Remove all the agglutination, or all the non-agglutination assays from the combined plate.*

Background light error	There is excess light in the reading compartment. *Check that the plate carrier door closes correctly. If it does close correctly but the problem persists, contact an approved service center.*
Barcode COM Error	A fault has occurred in the interface between the barcode scanner and the Barcode Program. *Switch the Reader off and on again. If the problem persists, contact an approved service center.*
Barcode Not Found	The barcode scanner was unable to read the barcode correctly. *Check that the barcode label is printed correctly, and that it is positioned correctly on the side of the microplate. Check that the Barcode Program has been set up to scan the type of barcode you are using. Refer to the Barcode Program User Manual. Try scanning other barcodes of the same and different types. If the problem persists, contact an approved service center.*
Barcode retry error **Barcode timeout error**	A fault has occurred in the interface between the barcode scanner and Revelation. *Switch the Reader off and on again. If the problem persists, contact the service center.*
Checksum Error in File	A data file has been corrupted. *If the file contains setup parameters or directory information, the Reader will rebuild the file whilst displaying a rebuilding message.* *If the file is a test, plate data, or a curve data file, you will have to recreate it.*
Command Time out	*Check the communication settings and cables. Contact the service center if the error persists.*

Curve File Error	The Reader could not find the file required. *Recreate the file.*
Curve Fitting Error !	An error has occurred when trying to calculate a curve fit. *Check your plate data and curve fit parameters.*
Device already open	*Check the communication settings and cables. Contact the service center if the error persists.*
Device not open	*Check the communication settings and cables. Contact the service center if the error persists.*
Diode selection error	*Contact an approved service center*
Directory File Error	The Reader could not find the directory file required. *The Reader will rebuild this file whilst displaying a rebuilding message.*
Divide by Zero Error	The Reader has found an error in an equation or test. *If you cannot find an error in the test, switch the Reader off and start again.*
DOMAIN Error	*Contact an approved service center.*
Error building DCB	*Check the communication settings and cables. Contact the service center if the error persists.*
Error in default parameters	*Contact the service center if the error persists.*
Error in Equation	The Reader has found an error in an equation. *Check all equations to make sure that they are valid, and edit them if necessary.*
Error Loading Module	The Reader cannot find an executable file. *Contact an approved service center.*
Error Opening File	The software could not find the file you asked for. *Recreate the file.*
Error setting comm state	*Check the communication settings and*

	cables. Contact the service center if the error persists.
Fatal Error! **Bad Drive Request Structure Length.** **Data Error (CRC Error).** **Drive Not Ready.** **General Failure.** **Read Fault.** **Sector Not Found.** **Seek Error.** **Unknown Command.** **Unknown Media.** **Unknown Unit.** **Write attempt.....** **Write Fault.**	The Reader has detected a problem with the floppy disk or the hard disk. There may be no disk in the floppy drive, or your floppy disk may be write protected, or corrupted. *Switch the Reader off and remove the floppy disk from the disk drive, if necessary. Switch the Reader back on. Place the floppy disk in the drive. If the disk is write protected, slide the tab back to the closed position.* *If the Reader cannot read your disk it may be corrupted. If this is the case, stop using the corrupted disk and copy your files from your backup disk onto a new disk.*
File Checksum Error	A data file has been corrupted. *If the file contains setup parameters or directory information, the Reader will rebuild the file whilst displaying a rebuilding message.* *If the file is a test, plate data, or a curve data file, you will have to recreate it.*
File Error	The software could not find the file you asked for, either because it was not there, or because of memory problems *Recreate the file if it does not exist. If this error occurred during a copy operation, switch the Reader off and restart it, then try again.*
File... is not the correct version	The file you asked for has been saved using a different software version. *Upgrade the software version of the program you are using. Contact an approved service center.*

Filter Motor Error 1 **Filter Motor Error 2**	The Reader cannot initiate the Filter Motor. *Check that number of filters specified in the Reader's Filter menu corresponds with the number of filters installed in the filter wheel and check that the filter wheel index holes are not blocked. Refer to the section on Changing the Optical Filters, in the Reader User Manual. If the problem persists, contact an approved service center.* *Make sure that the filters are installed in sequence in the filter wheel and that no slots have been skipped. Then check the software setup and make sure the correct number of filters have been specified.* *Filters get dirty and go bad over time. Wipe the filters with a lint free cloth, then run Calibration or Verification Plate to make sure that the filters are working properly. If a filter is bad, replace it.* *An incorrectly installed lamp or a faulty lamp may not be transmitting enough light. Make sure the lamp is properly installed (refer to "Fitting the Lamp and Optical Filters" in Chapter 2 in the Microplate Reader User Manual). If it is, change the lamp to see if that will resolve the problem.* *The optic assembly may not be pushed all the way inside the Reader. If you just changed the lamp, make sure it is fitted correctly and that the rear panel is back on.*

Filter n Bad	One of the filters is faulty or not installed, **n** refers to the filter number. *Check that the filters are installed in the correct filter holders. Check the filter for dirt or fingerprints, and clean it carefully using a lint-free cloth. If the problem persists, replace the filter.* *Refer to the section on Changing the Optical Filters in the Reader User Manual.*
Gain selection error	*Contact an approved service center.*
H8.....	An error has occurred in the temperature controller communications. *Contact the service center if the error persists.*
Hardware not present	The Reader is not correctly installed, or not set up correctly in the Configure Reader box. *Check that the Reader is installed correctly (refer to the Reader manual) and configured correctly.*
Incorrect Format	You have tried to load a test or data containing a chinese plate format, but the test or data expected a 12x8 plate format. *Change the plate format using the Settings/Configure Reader... option.*
Invalid Axes Mode	You have selected a Sigmoid (4PLC) curve type, with an invalid axis fit. *The Reader will change your axis fit to Semi-Log. Press any key to continue.*
Invalid byte size	*Check the communication settings and cables. Contact the service center if the error persists.*
Invalid Copy	You have tried to copy a test, and given the new test the same number as the original test. The original test has now been lost. *Re-write the test, and copy it using a different number.*

Invalid or unsupported ID	*Check the communication settings and cables. Contact the service center if the error persists.*
Invalid shake parameters	The settings you have entered for the User Shake facility are too high. *Choose a different combination of shake settings.*
MATH Error	*Contact an approved service center*
Mathematical Error in an equation	The Reader has found an error in an equation. *Check all equations to make sure that they are valid, and edit them if necessary.*
Module Not Loaded	You are trying to run an assay which contains operations not available for your Reader model. *Remove the unsupported operation.*
MRX error nnn	*Contact the service center if the error persists.*
No Bulb	The Reader cannot detect the bulb. *Check that the bulb is correctly connected and that it lights correctly. Replace it if necessary. Refer to "Fitting the Lamp and Optical Filters" in Chapter 2 in the Microplate Reader User Manual.*
Optic Channel n Bad	One of the optics channels is faulty, **n** refers to the channel number. *The Reader can still work but the readings from the column containing the faulty channel must be ignored. Contact the service center.*
Overflow Error	*Contact the service center*
Parallel Line Error	The parallel line fit contains too many lines *Reduce the number of lines in the parallel line fit by removing Test wells. Refer to the Parallel Line Bioassays section in the Endpoint Program User Manual.*

Password Error	The wrong password has been entered. *Enter the correct password. If you cannot remember the password, you will have to re-enter the test.*
Photodiode selection	*Contact the service center.*
Plate drive sensor error	*Try repeating your last command and contact the service center if the error persists.*
Plate File Error	The Reader could not find the file you asked for *Recreate the file.* *An incorrectly installed lamp or a faulty lamp may not be transmitting enough light. Make sure the lamp is properly aligned (refer to "Fitting the Lamp and Optical Filters" in Chapter 2 in the Microplate Reader User Manual). If it is, change the lamp to see if that will resolve the problem.* *The reference holes on the plate carrier, located at A1 and H12, may be dirty or obstructed. Blow air through the holes and remove any obstructions.* *Make sure the sprint clips are not bent.*
Plate Motor Error	The Reader cannot start the Plate Motor. *Check for obstructions to plate carrier movement and whether any of the plate carrier index holes are blocked, then press any key. If the plate carrier is stuck, you may carefully attempt to free it by hooking a suitable implement around the edge.*
Please enter a valid well location	You typed in a well location that does not exist. *Type in the correct well location.*
Please enter an integer between x and y	You have entered a value which is out of range. *Enter a valid value.*
PLOSS Error	*Contact an approved service center.*

Printer switched off **Printer Offline** **Printer Paper Empty**	The Reader has detected a problem with the printer. *If necessary select ABORT, the Reader will reset all your printer options, so you will have to set these again using the Setup menu, refer to the Setup menu section in the Reader User manual. Switch the printer on, press the on-line button or replace the paper, according to the specific instruction.*
Reader Overheat	*Contact an approved service center.*
Set point is below ambient + 4 ° C	The temperature controller is set to a temperature it cannot achieve. *Make sure the set temperature is above ambient + 4°C. Ambient is defined as the temperature in the thermal chamber. This temperature is higher than room temperature and increases with normal Reader activity, plate shaking, plate reading and other incubations. If you have been running a large number of readings or shake operations, leave the Reader to cool, or set a higher temperature. If you have just run a high temperature incubation, the Reader will only allow you to set the lower temperature if it is within range. However, you should leave the Reader to cool before inserting the plate.*
SING Error	*Contact an approved service center*
Slate Error	*Contact an approved service center.*
Test File Error	The Reader could not find the required file. *Recreate the file.*
The temperature controller cannot be found	An error has occurred in the temperature controller. *Contact the service center if the error persists.*

Timing overflow error	You have not allowed a sufficient time interval between kinetic readings or you have interrupted the kinetic assay. *Increase the time interval or try running the assay again, without performing any mouse or keyboard operations.*
TLOSS Error	*Contact an approved service center.*
Token error in an equation	The Reader has found an error in an equation. *Check all equations to make sure that they are valid, and edit them if necessary*
Too few Standards	The curve fit type you have specified requires more standards than you have entered. *Edit the test template to contain more Standards, or use a stored curve.*
Unable to allocate queues	*Check the communication settings and cables. Contact the service center if the error persists.*
Underflow Error	*Contact an approved service center.*
Unsupported baud rate	*Check the communication settings and cables. Contact the service center if the error persists.*
Warning! Fit aborted after 20 iterations	Data was a poor match for the defined Sigmoid (4PLC) curve. *Edit the plate to remove ODs which are a poor match, using the Outlier Removal option.*
Warning! Invalid blank mode selected. Blank mode has been modified	The blank mode you chose was not appropriate for the template. *Change the blank mode or change the template.*
Warning! Limits are out of sequence	The ++ positive, +++ positive or ++++ positive limits you have defined are not in sequence, relative to the positive and negative limits. *Change the limits so that they follow the sequence of the positive and negative limits.*

Warning! Multiple wavelength calculation error	The Multi-Wavelength mode calculation did not work correctly. *If you specified Well calculation mode, check that the well does exist on the template, and check that the OD for this well was not OVER. Check your data to make sure that the calculation mode specified could be used. Change the calculation mode if necessary.*
Warning! Nominal value is out of range	The dilution value you have set for the change between positive and negative threshold ranges was not correct within the +/–2 tolerance. *Run the test again using a different nominal value, or use the Titration Multiplier facility to correct the value.*
Warning! Plate ID does not match assay prefix/suffix format	The plate ID you entered, or the barcode scanned, does not match the tag specified in the test. *Check that the plate in the plate carrier is the one you wish to process. If this message was generated after a barcode scan, check that the barcode label is not damaged and check that the software is set up to recognise the correct barcode type.*
Warning! Quality Control Failed	The criteria for the defined QC equations have not been met.
Warning! Unable to calculate percentage response values	The Standard samples you have specified for use with this facility, either do not exist, or have not been included in the Curve Fit calculations. *Create or include the Standards, or change the Percent Response samples to ones which are already included in the calculations.*

Warning! Unable to contract template	The software was unable to reduce the template in the way you specified. Templates cannot be reduced if: • the original plate contained any empty wells • reducing the template means removing all the Test samples • the templates combined onto the plate had different row and column orientations. *Rectify the problem and try again.*
Warning! QC values are 0.000 – Check Data Matrix	There is a zero in the QC equation. *Verify if the OD value in the Data Matrix is a result of 0.000 OD or an "OVER." The Endpoint program will convert an "OVER" value to 0.000 OD in the QC equation.* *"OVER" readings result when well OD's exceed the dynamic range of the Reader.*

Section 2 Solving Problems

This section describes hardware, printing, and general problems and provides the recommended actions to solve them.

Hardware Problems

Hardware Problem	Recommended Action
The Reader keypad will not operate.	The Reader is operating under the control of an external computer. *Refer to the Microplate Reader User Manual for more information.*
The Reader powers up but does not access the disk drive.	The line voltage setting is incorrect or the Reader is defective. *Switch the Reader off and adjust the voltage setting. If the problem persists, contact the service center.*
The Reader powers up but only accesses the disk drive briefly.	The disk is corrupt or not one of the Reader's disks. *Try using the backup disk. If this does not work, contact an approved service center.*
Readings in rows F, G and H are unusually low.	Stray light may be entering the reading compartment. *Check that plate carrier door closes correctly.*
The hardware and software setup options have been lost.	*Verify that you have changed the floppy disk you were using. Setup options are stored with the software programs so every program you are running may have different setup option.*
Speaker beeps several times in succession on power up.	A fatal self-test error has occurred. *The Reader will probably crash. When it does, restart it and count the number of beeps which occur on power up. Contact an approved service center, informing them of the number of beeps.*

Printing Problems

Printing Problem	Recommended Action
The Reader produces an unrecognizable printout.	An incorrect printer driver is installed. *Verify that the driver in Setup menu corresponds to printer type; if not, contact an approved service center.*
Results are printed on the right of the page, overlapping each other.	Increase the page width using the Setup menu. Refer to the Setup menu section in the *Reader User Manual.*
The Reader reverts to No Printer mode each time it is switched on.	The Reader is being switched on before the printer *Switch the printer on before the Reader*
Parts of the output are overwritten and vertical lines appear on the screen.	Trim down the output by removing processing options, or, if you are using a number of tables, remove one or more of them.
On a tractor fed printer: the printout is not the correct place on the page.	*Position the printer paper so that the top of the page is just above the print head for correct page position.*
On a laser printer: The last page of any printout does not print automatically.	*Switch the printer off-line and select Form Feed.* *Switch the printer on-line before trying to print again.*

General Problems

General Problem	Recommended Action
A self-test is carried out on every read.	The Self-test Retest option is toggled on. *Set the Retest option to NO using the Setup menu.*
All readings are Over.	The Over limit is too low. *Reset the Over limit using the Setup menu.*
The Reader does not process the full 12x8 matrix.	The Plate Type option is set to Alternative Format. *Set the Plate Type option to Standard format using the Setup menu.*
You wish to recall plate data you did not assign a plate ID.	The Reader can store 100 sets of plate ID and it assigns a default plate ID numbered 00 to 99 for any plates which you do not assign an ID or a barcode. Once the Reader reaches capacity it starts overwriting plates stored under default IDs without informing you. *If the data you require has not been overwritten, you may be able to find it by printing the directory of plates. This will tell you when each set of data was obtained, so you can work out which plate ID you need.*

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PR Microplate Reader

87360
87364

Endpoint Program User Manual

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English

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Table of Contents

About This Manual 1

Chapter 1 Features and Specifications 3

Chapter 2 Understanding the Endpoint Program 5

2.1 Wavelength Modes 7
Single and Dual Wavelength Modes 7
Multiple Wavelength Mode 8
2.2 Optical Path 9
Effect of Pathlength 10
2.3 Blanking 10

Chapter 3 Installation 11

3.1 Installing Endpoint in a PR 1100 Reader 13
3.2 Re-Installing Endpoint in a PR 2100 Reader 14
3.3 Upgrading from a Previous Version 14
3.4 Upgrading Assay Protocol Files in the PR 2100 15

Chapter 4 Creating and Editing Tests 17

4.1 Creating a New Test – NEW 20
Test Number 20
Test Name 21
Plate ID Prompt 22
Shake Time and Shake Modes 22
Define Template Prompt 24
Defining the Template Manually 24
Defining the Template Automatically 29
Wavelength Mode 30
Blanking 33
Results Matrix 36
Area Statistics 36
Quality Control Equations 38
Inserting Output Options Into the Test 44
Threshold Matrix Output Option 45
Ratio Matrix Output Option 52
Curve Fitting Output Option 56
Difference Matrix Output Option 64
4.2 Editing a Test – EDIT 66
Copy 66
Editing Tests Options 67
4.3 Printing Tests – LIST 68
4.4 Printing Directories – DIR 69

Chapter 5 Running Tests and Recalling Plates 71

5.1 Running a Test 74
Test Number 74
Number of Samples 75
Plate ID 77
Barcode Scan 78
Reading 78
Variables 79
Editing Areas 79
Curve Fit Output 79
Read Again 83
5.2 Recalling Plates 83

Chapter 6 Utilities 85

The Utility Menu 87
Manual 88
Menus 89
Version 90
Backup 90
C.V. 91
Stats 91
Batch 92
Spectrum 92

Chapter 7 Computer Control 95

7.1 Hardware Specification 97
Linking with an IBM-Compatible PC 98
Linking with an Apple Macintosh 99
7.2 Software Specification 100
7.3 Handshaking 100
7.4 Checksum 102
7.5 Computer Control Commands 102
0 Step X/Y 103
1 Start/Stop 104
2 Read Mode 106
3 Filter Select 106
4 Blank Modification 107
5 Print Control 108
6 Display Control 110
7 Computer Control 110
9 Reduced Matrix 110
7.6 Data Format 111
Response with Data 111
Response without Data 113
Uncontrolled Transmission 113

Appendix A: Examples of Plate Formats 115

Single Well Standards in Rows 117
Single Well Standards in Columns 117
Double Well Standards in Rows, Replicates in Columns 118
Double Well Standards in Columns, Replicates in Rows 118
Triple Well Standards in Rows, Replicates in Columns 119
Triple Well Standards Columns, Replicates in Rows 119
Alternative Format: Single Well Standards in Rows 120
Alternative Format: Quadruple Well Standards in Columns, Replicates in Rows 120

Appendix B: Sample Printouts 121

Directory of Tests 123
Directory of Plates 124
Directory of Curves 125
Results Matrix: Labelled OD Matrix 125
Threshold Data: Test Printout 126
Threshold Data: Results Printout 127
Curve Fit Data: Test Printout 128
Curve Fit Data: Results Printout 129
Curve Fit Data 130
Linear Power Fit 130
Quadratic Power Fit 130
Curve Fit Data 131
Cubic Power Fit 131
Quartic Power Fit 131
Curve Fit Data 132
Cubic Spline Fit 132
Polygon Fit 132
Curve Fit Data 133
Sigmoid Fit 133
Michaelis-Menten Fit 133
Curve Fit Data 134
Akima Fit 134
Ratio Data: Test Printout 135
Ratio Data: Results Printout 136
Difference Data: Test Printout 137
Difference Data: Results Printout 138
Area Statistics: Test Printout 139
Area Statistics: Results Printout 139

Appendix C: Curve Fitting 141

Linear Regression 144
Polygon Fitting 145
Non-Linear Curve Fitting 146
Quadratic, Cubic and Quartic Regression 147
Cubic Spline Curve Fitting 148
Other Equations 149
Michaelis-Menten 149
Sigmoid Equation 151
Akima Curve Fit 153
Axis Fitting 155
Semi-Log Fit 155
Log-Log Fit 155

Index 157

About This Manual

This manual has been written for assay developers and describes the features and concepts of the Endpoint Program.

With the information in this manual, you can:

- Create, edit and run assay test procedures
- Manipulate the results of tests using standard thresholding and curve fitting methods.

This manual assumes that:

- The Reader has been installed according to the instructions in the *Microplate Reader User Manual*
- You are familiar with using the Reader user interface
- You have some degree of numerical aptitude
- You have a basic understanding of how to write assays, and a basic knowledge of Enzyme Linked Immuno Sorbent Assay (ELISA) techniques.

This manual does not attempt to teach you how to write meaningful assays, as it confines itself to a description of the program features.

For the following information, refer to the *Microplate Reader User Manual*:

- Safety information
- Installation and configuration instructions
- Maintenance instructions.

For explanations of error messages and possible solutions, refer to the *Troubleshooting Guide*.

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Chapter 1 Features and Specifications

The Endpoint Program is designed for use with the Microplate Reader.

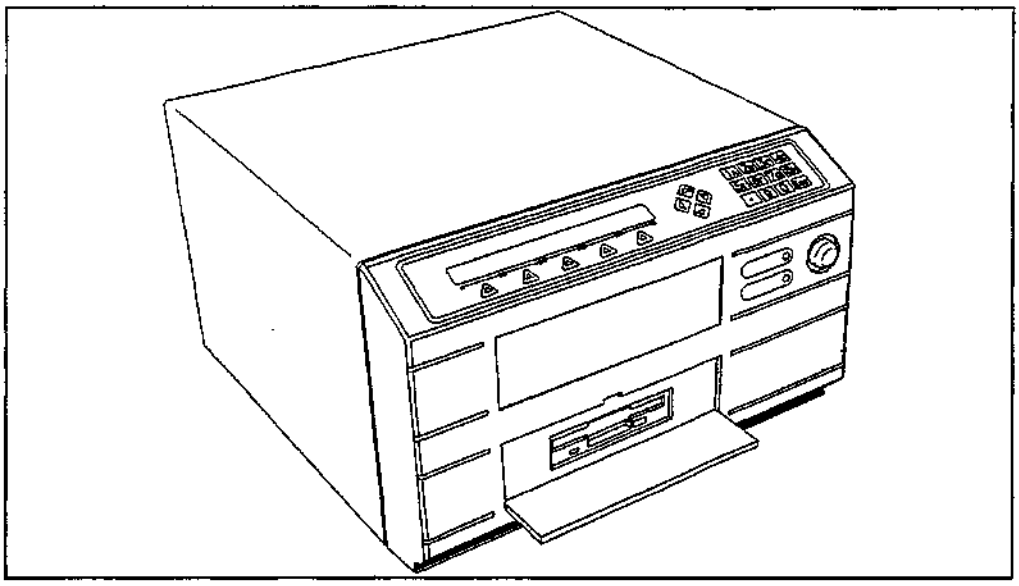

Figure 1 The Microplate Reader

The features and specifications of the Reader can be found in the *Microplate Reader User Manual*. The features and specifications of the Endpoint Program are as follows:

Menu language	English/French/German/Italian/Spanish	
Blanking	Air Paired Wells Individual Wells Average Whole Plate Average	Last Plate Row Column Each well on the plate
Clock function	Day, date and time	
Plate identification	Manual Barcode option	
Tests per plate	12	
Short Cut Test Selection	Eight function keys available	

Data storage	3.5" 1.44Mb floppy disk or optional hard disk drive Storage of up to 100 tests, 100 plates and 20 curves
Program Load Time	Using a PR 1100 Reader <40 seconds Using a PR 2100 Reader <1 second
Wavelength Modes	Single Dual Multiple
Results Output	Optical Density (OD) data matrix Threshold, Difference and Ratio matrices. Graphical Curve Fit output: Spline Polynomial regression – linear, quadratic, cubic and quartic functions Point-to-point straight line fitting Michaelis-Menten equation Sigmoid (4PLC) Parallel Akima fit Axis Fits: Linear Semi-log Log-log Auto function (for regression coefficient) Echo of calculated results to RS232

Chapter 2 Understanding the Endpoint Program

2.1 Wavelength Modes 7
Single and Dual Wavelength Modes 7
Multiple Wavelength Mode 8
2.2 Optical Path 9
Effect of Pathlength 10
2.3 Blanking 10

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This chapter describes certain aspects of the theory of photometry, including wavelength selection, optics and blanking. These processes are described simply so as to give you the basic information necessary to create effective microplate tests.

2.1 Wavelength Modes

The Reader is able to take readings in three different modes:

- Single--using one test filter
- Dual--using one reference and one test filter
- Multiple--using a combination of filters

Single and Dual Wavelength Modes

Using dual wavelength mode can reduce errors caused by dirt and scratches on the bottom of the wells, but single wavelength mode is sufficient for most applications.

The choice of test and reference filter wavelengths depends on the particular enzyme/substrate system being tested. However, the following rules should usually be followed:

1. The **test filter** wavelength should be at or near the maximum absorbance of the reaction product.
2. The **reference filter** wavelength should lie outside the absorbance band of the system but not far removed.

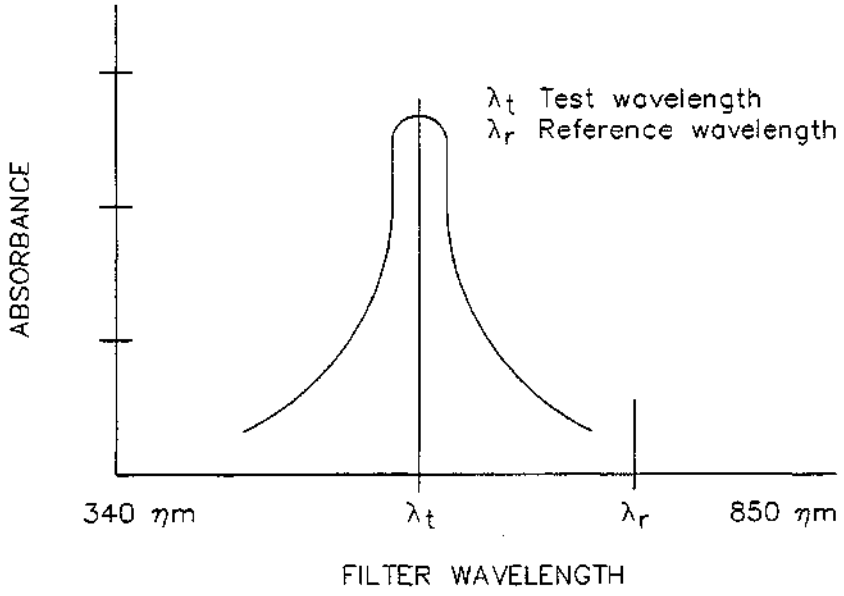

Figure 2 Dual Wavelength Selection

The Reader subtracts the absorbance at the reference wavelength (λr) from the absorbance at the test wavelength (λt) to minimize the effect of systematic errors.

- λr is the wavelength lying outside the major absorbance band of the sample but not far removed from λt
- λt is the wavelength at or close to the peak absorbance of the reaction product.

If a test requires particular accuracy, you may specify test and reference filters of the same wavelength. The Reader will average the ODs produced using each filter, giving a more accurate result.

Note: If you have problems choosing filters, use the Spectrum option to recommend wavelength. Refer to the Spectrum section in Chapter 6.

Multiple Wavelength Mode

Using multiple wavelength mode can help you to examine results which would normally be outside the range of the Reader. It lets you take two readings of each sample using different filters.

The first reading is run in **Primary mode** using standard filter wavelengths, this can be run in single or dual wavelength mode. The second reading is run in **Secondary mode** using slightly higher or lower wavelengths, this can also be run in single or dual wavelength mode.

The Reader automatically uses the primary readings when it calculates its results. If, however, any of these ODs are above the Over limit of the Reader, it then examines the secondary readings of the wells in question.

The secondary ODs, will be in a different range from the primary readings (because they were obtained using different filters) so they must be converted into the same range as the primary readings. To achieve this the Reader offers three different calculation modes.

1. **Linear Regression** calculates a line of best fit between the two sets of data. This line is used to convert secondary ODs into the correct range.
2. **Well Location** uses a well specified by the user to convert the readings. The ratio between the primary and secondary ODs from this well is applied to the other secondary ODs.
3. **Constant Value** uses a constant specified by the user. The secondary ODs are multiplied by this value to convert them to the correct range.

2.2 Optical Path

Figure 3 is a schematic diagram illustrating the optical path through the Reader. A tungsten halogen lamp projects a light beam horizontally through a heat absorbing filter. This beam is focused and passes through a filter located in the filter wheel. Here the beam is separated into 13 channels. One of these channels is used as a reference channel to monitor the light output of the lamp. The other 12 beams are directed upwards through a row of 12 wells on the microplate, onto an array of silicon photodiodes. The photodiodes accurately measure the intensity of light transmitted through each well.

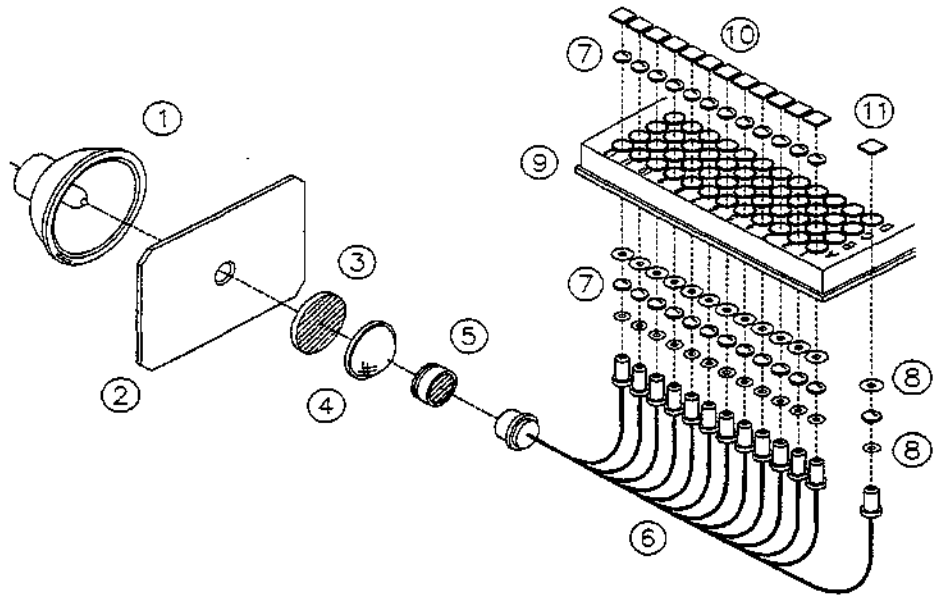

Figure 3 Reader's Optical Path

1	Lamp	6	Optic Fibres
2	Field Stop	7	Lenses
3	Heat Filter	8	Optic Stops
4	Lens	9	Microplate
5	Filter	10	Photodiodes
		11	Reference Diode

Effect of Pathlength

Light absorption by a material is determined by the Beer-Lambert law. This states that the:

> **Absorbance of Light** is directly proportional to the product of **Pathlength** and **Concentration**.

- **Pathlength** – is the distance in cm which the light beam travels through the absorbing material.
- **Absorbance** – is usually expressed in terms of a standard cell of 1cm pathlength.
- **Concentration** – is expressed as moles per litre of fluid.

The pathlength through a solution in a microplate well is less than 1 cm. But accurate values of absorbance can be obtained as long as equal volumes of Blanks, Standards and Test samples are dispensed into wells of equivalent size and shape.

2.3 Blanking

The Reader lets you subtract a reference value from all the ODs. It automatically uses air as a reference, but for certain applications other reference levels may be more appropriate.

For example, you may want to eliminate the absorbance of a reagent solution from the test result. The Reader can hold the OD of this reagent solution in memory and subtract it from all subsequently read ODs.

Blanks may be single wells, or an average of wells.

Chapter 3 Installation

3.1 Installing Endpoint in a PR 1100 Reader 13
3.2 Re-Installing Endpoint in a PR 2100 Reader 14
3.3 Upgrading from a Previous Version 14
3.4 Upgrading Assay Protocol Files in the PR 2100 15

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CAUTION **This installation procedure assumes that the Reader has been installed correctly and that the voltage selector is on the correct setting. (Refer to the *Microplate Reader User Manual* for instructions.)**

Note: Before installing the Endpoint Program, make a working copy of the Endpoint disk, and store the original disk safely. The copy can be made on an external PC, using Windows ***File Manager****, or the MS DOS* ***diskcopy*** *command.*

3.1 Installing Endpoint in a PR 1100 Reader

Note: If you are using the Endpoint Program disk for the first time, it will contain all the default hardware and software setup options. To reset these options, refer to Chapter 2 of the Microplate Reader User Manual.

1. Switch the Reader off.
2. If the Reader was already running a software program, remove the original disk from the floppy disk drive.
3. Insert the Endpoint working disk into the floppy disk drive.
4. Switch the Reader on.

When the Reader has completed its self-test routines (refer to the *Microplate Reader User Manual* for details), the Main menu appears, showing the Endpoint option.

```
READY              6:65P  30.006.94
ENDPOINTSETUP
```

To access the Endpoint Program press the ∧ key below the **ENDPOINT** option.

The Reader will now run the Endpoint Program until you insert a different software program disk.

3.2 Re-Installing Endpoint in a PR 2100 Reader

The Endpoint Program is supplied pre-installed on the PR2100 hard disk drive. In the event that the software is accidentally deleted or one or more files have been corrupted, you will need to re-install the software.

To re-install the software:

1. Locate the Endpoint Program disk which was originally supplied with your PR 2100 Reader.
2. Insert the disk into the PR 2100 Reader disk drive and turn on the instrument.

   The software is automatically installed and the Main menu appears on the display:

READY	6:65P 30.006.94
ENDPOINT	SETUP

3. Remove the disk from the drive and return it to its storage place for safekeeping.

Note: Your assay protocol files and plate data will not be affected by the re-installation procedure.

3.3 Upgrading from a Previous Version

If you receive an updated version of the Endpoint Program, use the following procedure to upgrade the Reader.

Note: This upgrade procedure will not affect tests you have created previously. These tests are fully compatible with the new software version.

To upgrade from a previous version:

1. Switch the Reader on. It will carry out its self-tests (refer to the *Microplate Reader User Manual* for details), and then display the Main menu. The appearance of this menu will depend on which software options are installed, but the **SETUP** option will always be present. For example:

READY	4:45P 30.06.94
ENDPOINT	SETUP

2. Select **SETUP** and then **OTHER** to access the second Setup menu.

SETUP ?	4:45P 30.06.94
MAINT COMMS MEMORY PARAM	CE

3. Select **MEMORY** from the setup options. The Reader will ask if you want to reset the memory or to upgrade the Reader software.

SETUP MEMORY ?	4:45P 30.06.94
RESET UPGRADE	CE

4. Select UPGRADE. The Reader will prompt you to insert the Upgrade working disk and then the Endpoint disk if you are using a PR 1100 Reader.

5. As the Reader is upgrading your disk, it will come across filenames which are already being used on your disk. When this happens it will display a message asking if you want to overwrite the old file.

   If your disk contains pre-defined PR Assay Data files: You may have changed some of the pre-defined assay, data or curve files, and want to keep the changes rather than overwrite them. Select **YES** to overwrite files you do not require (for example, TXT, EXE and INF files, plus any assay, curve and data files that you do not need). Select **NO** to leave intact any files you want to keep. If you do not want to keep any of the pre-defined assays, select **ALL** to overwrite them all with the new assays files.

   If your disk does not contain pre-defined PR Assay Data files: The upgrade disk will only overwrite the redundant executable and text files, so you should select **ALL** to upgrade to the new version.

   This will not affect the Reader's ability to open files created in a previous version on the software.

6. When the upgrade is complete, remove the disk from the disk drive. You can access the Endpoint Program in the usual way.

3.4 Upgrading Assay Protocol Files in the PR 2100

From time to time, an assay protocol file upgrade disk may be supplied. This disk will upgrade the library of assays available on your PR 2100 hard disk drive.

To install the upgrade disk:

1. Switch the Reader on. It will carry out its self-tests (refer to the *Microplate Reader User Manual* for details), and then display the Main menu. The appearance of this menu will depend on which software options are installed, but the **SETUP** option will always be present. For example:

READY	4:45P 30.06.94
ENDPOINT	SETUP

2. Select **SETUP** and then **OTHER** to access the second Setup menu.

```
SETUP ?              4:45P 30.06.94
MAINT  COMMS  MEMORY  PARAM    CE
```

3. Select **MEMORY** from the setup options. The Reader will ask if you want to reset the memory or to upgrade the Reader software.

```
SETUP MEMORY ?       4:45P 30.06.94
 RESET  UPGRADE             CE
```

4. Insert the upgrade disk in the drive and select UPGRADE. The assay protocol files are copied to the hard drive.
5. When the Reader has finished the process, press the CE key several times to return to the Main menu.
6. Remove the upgrade disk from the disk drive.

Chapter 4 Creating and Editing Tests

4.1 Creating a New Test – NEW 20
Test Number 20
Test Name 21
Plate ID Prompt 22
Shake Time and Shake Modes 22
Define Template Prompt 24
Defining the Template Manually 24
Defining the Template Automatically 29
Wavelength Mode 30
Blanking 33
Results Matrix 36
Area Statistics 36
Quality Control Equations 38
Inserting Output Options Into the Test 44
Threshold Matrix Output Option 45
Ratio Matrix Output Option 52
Curve Fitting Output Option 56
Difference Matrix Output Option 64
4.2 Editing a Test – EDIT 66
Copy 66
Editing Tests Options 67
4.3 Printing Tests – LIST 68
4.4 Printing Directories – DIR 69

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Once the instrument is switched on and the self-test routines are successfully completed, the Main menu appears. This shows the choice of programs.

The appearance of this menu will depend upon the application programs installed. If the Endpoint Program is the only software program installed the Main menu will show only the Endpoint and Setup options.

```
READY                4:45P  30.06.94
ENDPOINT                      SETUP
```

Select **ENDPOINT** to view the Endpoint menu.

```
ENDPOINT READY        4:45P  30.06.94
 START  RECALL  UTIL  PROGRAM  CE
```

- **START** Lets you run assay test procedures which have been created using the Program menu.
- **RECALL** Lets you recall data obtained from a previous assay test procedure for further processing.
- **UTIL** Lets you carry out a manual operation, display the software version number, define calculation modes and set menus.
- **PROGRAM** Lets you create and edit assay test procedures.

While the Main menu or Endpoint menu is displayed, the plate carrier can be moved in and out using the up and down cursor keys. The up key moves the plate carrier in; the down key moves it out.

Note: If short menus have been selected using the Util menu, up to 8 tests may have been given short-cut keys. These will appear at the ENDPOINT READY display, for example:

```
ENDPOINT READY        4:45P  30.06.94
TEST1  TEST2  TEST3  TEST4  ENDPOINT
```

The left and right (< >) cursor keys may be used to scroll along the list of tests, and to access tests five to eight. Refer to the Menus section in Chapter 6.

4.1 Creating a New Test – NEW

To create a new assay test procedure or edit an existing one, select **PROGRAM** from the Endpoint menu. The Program menu appears.

```
ENDPOINT READY        4:45P 30.06.94
 NEW   EDIT   LIST    DIR    CE
```

- **NEW** Creates a new assay test procedure.
- **EDIT** Modifies or copies an existing assay test procedure.
- **LIST** Prints all the steps in an assay test procedure.
- **DIR** Prints directories of tests, plates or curves.

When you create a new test, you:

- Assign a test number, test name, plate ID, shake time, and shake mode
- Define the template (manually or automatically)
- Select wavelength and blanking modes
- Specify whether you want to print a results matrix, include area statistics and quality control equations
- Specify the output options.

Select **NEW** from the Program menu to create a new assay test procedure.

Test Number

The Reader requests an test number.

```
TEST (00)             4:45P 30.06.94
              DIR   ENTER   CE
```

Use the keypad to enter a number, or use the cursor keys to increment and decrement the number. The up and down cursor keys change the number in single units. The left and right cursor keys change the number in tens.

If you enter the number of an existing test, the test name is displayed next to the number. The Reader will ask if you want to overwrite the existing test, for example:

```
CLEAR TEST RUBELLA    4:45P 30.06.94
 YES    NO                  CE
```

Select **NO** to leave the test intact and return to the Program menu. Select **YES** to overwrite the test.

Password Protected Tests

If the test you want to overwrite is protected by a password, you must enter the password before the Reader will overwrite it.

```
PASSWORD =          4:45P 30.06.94
    YZ ABCD        ENTER   CE
```

Passwords are up to 12 characters in length and may consist of upper- and lower-case letters, symbols, numbers and a decimal point. These are entered using the display and the alphanumeric keypad. Refer to the Password section later in this chapter.

If the password is correct, the Reader overwrites the test and asks you to enter the test name. If the password is incorrect, a **PASSWORD ERROR**! message is displayed and the display returns to the Endpoint menu.

Test Name

Once a valid number has been entered, the Reader requests a name for the test.

```
NAME =              4:45P 30.06.94
    YZ ABCD        ENTER   CE
```

Test names may be up to 12 characters in length and consist of the following characters:

- Numbers 0–9 and a decimal point.
  These are entered using the alphanumeric keypad.
- Upper-case letters, lower-case letters and symbols.
  Enter these as follows:

  a) Choose upper-, or lower-case letters or the symbols using the up and down cursor keys (∧ ∨)

  a) Scroll through the characters on the display with the left and right cursor keys (< >). Enter the character shown by the flashing cursor by pressing the (∧) function key below it.

Example

To assign the name TESTA4 to test number 4:

1. At the **TEST (00)** prompt select 04 and then select **ENTER**.
2. At the **NAME** = prompt press the < or > key till the letter **T** is indicated by the flashing cursor; press the ∧ key to enter the letter. Move to the letter **E** with the < key and press the ∧ key to enter the second letter.
3. Repeat this process for the other letters until the name is complete and key in the number 4 on the keypad.
4. Select **ENTER** to record the name.

Plate ID Prompt

If a plate ID prompt is inserted into the assay test procedure, the Reader will ask you to enter a plate ID when the plate is read.

```
PLATE ID PROMPT ?      4:45P  30.06.94
 YES    NO    BARCODE          CE
```

- **YES** At runtime, the Reader will ask you to enter a plate ID prompt. This ID will be used to label the unprocessed plate data for storage, and you can use it to recall the plate data if necessary.
- **NO** If you do not assign an ID, the Reader will assign a default one. As the Reader has a total capacity of 100 plates, default IDs are numbered 0000-0099, unless you have assigned IDs of your own. In this case there will be less default IDs available. Once the Reader reaches its 100 plates capacity it reuses all the available default IDs, overwriting the plate data they hold. It begins at 0000 and works upwards until all the default IDs are used up again. It does not inform you when it begins overwriting old plate data.
  If you intend to recall plate data at a later date you should always insert a plate ID prompt or a barcode scan.
- **BARCODE** At runtime, the Reader will look for a barcode on the side of the microplate. To use this option, make sure that you have set up the Barcode software in the Main menu (refer to the *Barcode User Manual*).
  This option only appears if the Barcode software is installed.

Shake Time and Shake Modes

The Reader asks if you want to specify a shake time.

```
SHAKE =              4:45P  30.06.94
                    ENTER   CE
```

If you insert a shake, the Reader will shake the plate for the required time before reading it. This will ensure that the reagents in the wells are completely mixed.

To insert a shake, enter a number of seconds and select **ENTER**. Minimum: 1 second, maximum: 59 seconds.

If a shake is not required, enter 0 seconds and select **ENTER**.

If you have specified a shake time, the Reader asks you to specify a shake mode:

```
SHAKE MODE ?          4:45P 30.06.94
  1     2     3    USER    CE
```

Select **1**, **2** or **3** to use one of the standard shake modes, or select **USER** to specify your own shake parameters.

- **Shake mode 1** — The Reader will shake the plate for the specified time at a frequency of 14Hz (+/– 1Hz) and an amplitude of 1.7mm.
- **Shake mode 2** — The Reader will shake the plate for the specified time at a frequency of 9Hz (+/– 1Hz) and an amplitude of 3.0mm.
- **Shake mode 3** — The Reader will shake the plate for the specified time at a frequency of 8Hz (+/– 1Hz) and an amplitude of 3.0mm.
- **User shake mode** — Intended for experimental purposes only and will not produce the exact amplitude and frequency specified. It will, however, produce reproducible shaking from machine to machine.

If you select **USER,** The Reader asks you for the amplitude of the shake.

```
AMPLITUDE (mm) =      4:45P 30.06.94
                   ENTER   CE
```

Enter a value for amplitude and select **ENTER**.
Minimum: 1mm, maximum: 10mm.

The Reader asks you for the frequency of the shake.

```
FREQUENCY (Hz) =      4:45P 30.06.94
                   ENTER   CE
```

Enter a value for frequency and select **ENTER**.
Minimum: 5Hz, maximum: 20Hz.

If the plate carrier is heavily loaded and the amplitude or frequency is very high, the plate carrier may stall. In this case, press the Esc key to reset the Reader. Always test run user shake mode settings before using them in actual tests.

Define Template Prompt

The Reader asks whether you want to define the template manually or automatically.

```
DEFINE TEMPLATE ?      4:45P 30.06.94
 YES   AUTO                    CE
```

- **YES** — You must input the locations of each well type manually.
- **AUTO** — The Reader automatically assigns well locations once you have specified the number and format of the wells.

To define the template automatically, refer to the Defining the Template Automatically section later in this chapter.

Defining the Template Manually

Select **YES** at the Template menu to access the Manual Template menu. The Reader asks which well type you want to assign to well location A1.

```
A1 =                  4:45P 30.06.94
B/S/T/C  USER  UTILITY FINISH    CE
```

- **B/S/T/C** — Assigns the well location to a well type: **B**lank, **S**tandard, **T**est or **C**ontrol.
- **USER** — Lets you create well types and then assign these to wells.
- **UTILITY** — Displays a Utility menu of tools which help you to define the template.
- **FINISH** — Saves the template.
- **CE** — Returns the display to the Template menu.

B/S/T/C Wells

Select **B/S/T/C** to assign **B**lank, **S**tandard, **T**est or **C**ontrol wells.

```
A1 =                  4:45P 30.06.94
 B1    S1    T1    C1     CE
```

Select **B1**, **S1**, **T1** or **C1** to assign well location A1 to a **B**lank, **S**tandard, **T**est or **C**ontrol well. If you want to start with a different well location you can scroll through the wells using the cursor keys.

Replicates

Each sample of a well type can be arranged in groups of one to four replicates. You can specify that each sample of a well type will contain the same number of replicates or that some samples will contain different numbers of replicates (mixed replicates):

```
B WELLS IN          4:45P 30.06.94
          MIXED  ENTER   CE
```

If you want to assign all the samples of this well type in the same number of replicates, type in the number of replicates on the keypad and select **ENTER**. If you want to assign some samples in different numbers of replicates, select **MIXED** and then select **ENTER**.

If you select mixed replicates, the Reader will ask you for the number of replicates in the first sample after you have specified the orientation of the replicates and the well type.

Orientation of Replicates

If you selected more than one replicate, or mixed replicates, the Reader asks if the replicates are to be positioned along rows, down columns or randomly.

```
REPLICATES IN       4:45P 30.06.94
COLUMNS  ROWS  RANDOM         CE
```

- **COLUMNS** Positions all replicates down the columns.
- **ROWS** Positions all replicates along the rows.
- **RANDOM** Lets you position all replicates randomly.

The orientation of the replicates applies to all the samples in the well type, even if the samples are arranged in mixed replicates.

Well Type Orientation

The Reader asks whether the next well of that type is to be placed in the next available space down the column or along the row.

```
B WELLS IN          4:45P 30.06.94
 COLUMNS  ROWS              CE
```

Select **COLUMNS** to position the next well in the next space working down the columns. Select **ROW** to position the next well in the next space working along the rows. This determines the next well location offered by the B/S/T/C menu.

Number of Mixed Replicates

Each time you select a well type which has been assigned in mixed replicates, the Reader will ask you how many replicates you want to assign to this sample. For example:

```
G10 =               4:45P 30.06.94
 B1X1   B1X2   B1X3   B1X4    CE
```

Select the number of replicates required for this sample.

The Reader returns to the B/S/T/C menu and offers you the next available well location. If you want to move to a different well location, you can do so using the cursor keys.

Example

All Test samples must be arranged in replicates of four which must be positioned along the rows. Each new group of Test well replicates must be positioned down the columns. The first well must be located in well A1. In total six groups of Test wells must be placed on the template.

At the Template menu select **YES** to manually define the template. The Reader will ask which type of well is required:

```
A1 =              4:45P 30.06.94
B/S/T/C  USER   UTILITY FINISH    CE
```

Select **B/S/T/C** to assign Test wells:

```
A1 =              4:45P 30.06.94
 B1     S1     T1     C1     CE
```

Select **T1** to place the first Test well in location A1. The Reader will ask for the number of replicates:

```
T WELLS IN          4:45P 30.06.94
          MIXED   ENTER    CE
```

To define Test wells in groups of four replicates, type **4** in using the keypad and select **ENTER**. The Reader will ask for the orientation of the replicates:

```
REPLICATES IN         4:45P 30.06.94
COLUMNS  ROWS   RANDOM          CE
```

Select **ROWS** to position the replicates along the rows. The Reader will ask for the orientation of subsequent groups of Test wells:

```
T WELLS IN          4:45P 30.06.94
COLUMNS  ROWS                 CE
```

Select **COLUMNS** to position subsequent groups of Test wells in the next available space down the columns.

The display will return to the B/S/T/C menu displaying the next available well location. This well location will be B1 because **COLUMNS** was defined in well type orientation, and the next available well location (working down the columns) is B1.

```
B1 =              4:45P 30.06.94
 B1     S1     T2     C1     CE
```

Select **T2** to position the next group of Test well replicates. All the variables of test wells have now been set so the Reader will not ask for replicates or orientations. The display will return again to the B/S/T/C menu displaying C1 as the next available well location. Continue to select Test wells until well location G1 and Test well T7 are displayed.

User Wells

Select **USER** from the Manual Template menu to create new well types and assign these to wells.

```
A1 =                4:45P 30.06.94
 NC1    PC1    CO1    PR1     CE
```

The four well labels displayed correspond to User well types one to four. The default names for these are:

NC Negative Control

PC Positive Control

CO Cut-off Control

PR Positive Reference

These names can all be changed to match your specific requirements.

To change the name of one of the well labels:

1. Select **NC1** to change the label of the first user well. The Reader will display the number and label of the well type.

```
USER WELL 1 = NC      4:45P 30.06.94
                   ENTER   CE
```

2. Select **ENTER** to confirm the name displayed, or **CE** to change it. The Reader asks you to enter a new label.

```
USER WELL 1 = NC      4:45P 30.06.94
    YZ ABCD         ENTER   CE
```

3. Use the left and right cursor keys to scroll through the characters. Press the function key to enter a character or **CE** to delete a character Use the alphanumeric keypad to enter numbers. User well names may be up to three characters in length. When the label is complete, select **ENTER** to accept it.

User wells are positioned in the same way as for Blank, Standard, Test and Control wells. Refer to the B/S/T/C Wells section earlier in this chapter.

Utility

Select **UTILITY** from the Manual Template menu to access the Utility menu.

```
A1  = S1.1          4:45P 30.06.94
 RESET DELETE  RENUM  FILL     CE
```

The Utility menu will display the first occupied well, followed by a list of tools.

- **RESET** Clears all the existing well assignments, allowing you to redefine the template from scratch.
- **DELETE** Deletes the currently displayed well assignment. The cursor keys can be used to scroll through the well locations.
- **RENUM** Renumbers all well types into unbroken sequences. This is useful if the plate has been edited and some wells have been removed leaving gaps in the numbered sequence.
- **FILL** Completes the rest of the template once the locations of specified wells have been defined.
- **CE** Returns the display to the Manual Template menu.

Once all the required well types have been specified, the remaining empty wells can be assigned to Test wells using the **FILL** command. If no Test wells have yet been specified, the Reader will ask for the number of replicates and the orientation. Refer to the discussions of Replicates and Orientation of Replicates earlier in this section. When the template is finished, select **CE** to return to the Manual Template menu, and then select **FINISH**.

Finish

Select **FINISH** from the Manual Template menu to end the template option. The Reader asks you to confirm that the template is correct.

```
TEMPLATE CORRECT ?    4:45P 30.06.94
 YES  LIST              CE
```

Select **YES** to accept the template, **LIST** to print the template or **CE** to return to the Manual Template menu, allowing you to edit the template.

☞ *Note: You should always print out a copy of the template before running the test to make sure that it is correct.*

Example templates are shown in Appendix A.

Defining the Template Automatically

Select **AUTO** from the Template menu to access the Auto Template menu. Only Standards and Test wells may be defined in this mode. The Reader asks you to define the Standards and then the Tests.

The display requests the number of Standards.

```
NO. OF STDS =          4:45P  30.06.94
                    ENTER   CE
```

Enter the number of standards required
Minimum: 1, maximum: 24.

Format

Two types of format can be used with the Reader, the standard 8 by 12 format or the alternative 4 by 10 format. The Reader asks whether or not you are using the alternative format.

```
ALTERNATIVE FORMAT ?   4:45P  30.06.94
 YES    NO                    CE
```

Select **YES** to specify alternative format, or **NO** to specify the standard format.

Replicates

Standards and Test wells can be arranged in groups of one to four replicates. These replicates are averaged into a single value. The Reader will ask how many replicates the wells must be arranged in. For example:

```
S WELLS IN             4:45P  30.06.94
                    ENTER   CE
```

Type in 1, 2, 3 or 4 using the keypad and select **ENTER**.

Orientation of Replicates

If a well type is arranged in replicates you must indicate whether these replicates are to be positioned along rows or down columns.

```
REPLICATES IN          4:45P  30.06.94
COLUMNS  ROWS                 CE
```

- **COLUMNS** Positions replicates down the columns.
- **ROWS** Positions replicates along the rows.

Well Type Orientation

The Reader then asks whether the next well is to be positioned down the column or along the row.

```
T WELLS IN          4:45P  30.06.94
                  ENTER    CE
```

Enter replicates desired for test.

```
S WELLS IN          4:45P  30.06.94
 COLUMNS  ROWS                CE
```

Select **COLUMNS** to position the next well in the next available space down the column, or select **ROW** to position the next well in the next available space along the row.

☞ *Note: Test wells are entered just as standards are entered..*

When Test wells and Standards have been specified, the Reader assigns all the wells automatically. The Reader will then ask you to confirm that the template is correct.

```
TEMPLATE CORRECT ?    4:45P  30.06.94
  YES   LIST                  CE
```

Select **YES** to accept the template, **LIST** to print the template, or **CE** to return to the Auto Template menu, allowing you to edit the template.

☞ *You should always print out a copy of the template before running the test to make sure that it is correct.*

Example templates are shown in Appendix A.

Wavelength Mode

The Reader asks which wavelength mode is required. The plate may be read in Single wavelength mode (with a test filter only), in Dual wavelength mode (with a test filter and a reference filter) or in Multiple wavelength mode (with a combination of filters). Refer to the Wavelength Modes section in Chapter 2.

```
WAVELENGTH MODE       4:45P  30.06.94
SINGLE  DUAL  MULTIPLE          CE
```

Single Mode

Select **SINGLE** to use a test filter only. The Reader asks for the test filter wavelength.

TEST FILTER 410NM	4:45P 30.06.94
NEXT LAST	ENTER CE

Use the **NEXT** and **LAST** commands to scroll through the list of filter wavelengths. Select **ENTER** to accept the displayed wavelength, or **CE** to return to the Wavelength Mode menu.

Dual Mode

Select **DUAL** to use reference and test filters. The Reader asks you to enter a test filter wavelength in the same way as for Single mode above. It will then request the reference filter wavelength.

REF. FILTER 550NM	4:45P 30.06.94
NEXT LAST	ENTER CE

Select the reference filter wavelength in the same way as you selected the test filter wavelength.

Note: If the test filter wavelength is the same as the reference filter wavelength, ODs are averaged instead of subtracted. Refer to Wavelength Modes in Chapter 2.

Multiple Mode

Multiple wavelength mode lets you take a primary and secondary reading for every sample using different filter wavelengths. The primary reading is normally used, but the secondary reading will be used if any well ODs are above the Reader's Over limit. The Reader uses a calculation mode chosen by the user to convert secondary readings into the primary reading range. Primary and Secondary modes can be both be run in dual or single wavelength mode.

For further information, refer to the Wavelength Modes section in Chapter 2.

The Reader asks which wavelength mode you want to use for the primary reading.

PRIMARY MODE	4:45P 30.06.94
SINGLE DUAL	CE

Select **SINGLE** or **DUAL** mode, and then select the filter wavelengths by scrolling through the available filters (refer to the sections above).

The Reader asks which wavelength mode you want to use for the secondary reading.

```
SECONDARY MODE          4:45P 30.06.94
SINGLE  DUAL                  CE
```

Select **SINGLE** or **DUAL** mode, and then select the filter wavelengths as before.

Once you have selected the filter wavelengths, the Reader asks which calculation mode to use, to convert secondary readings to the primary reading range.

```
CALCULATION MODE        4:45P 30.06.94
LINEAR  WELL  VALUE           CE
```

These modes are described below. For more information on these modes refer to the Wavelength Modes section in Chapter 2.

LINEAR converts the secondary readings using a line of best fit, calculated from the two sets of data.

WELL converts the secondary readings using the ratio between the primary and secondary ODs of a specified well. The Reader will ask you which well type you want to use (**B**lank, Standard, **T**est or **C**ontrol).

```
WELL =                  4:45P 30.06.94
 B     S     T     C     OTHER
```

Select a well type. The Reader will ask you for the number of the well. For example:

```
WELL = B                4:45P 30.06.94
                      ENTER   CE
```

Enter the well number using the keypad and press **ENTER**.

☞ *Note: You cannot specify a well type and number which you have not defined on the template.*

VALUE converts the secondary reading using a value which you enter using the keypad. The secondary readings required will be multiplied by this factor. The Reader will ask you for the value.

```
VALUE =                 4:45P 30.06.94
                  +/-   ENTER   CE
```

Enter the value using the keypad and select **ENTER**.

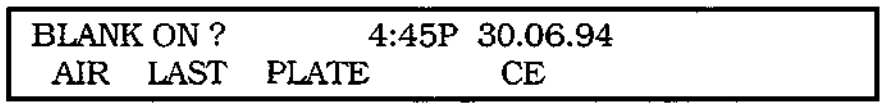
Blanking

The Reader asks which blanking mode you require. It automatically uses air as a reference, but for certain applications other reference levels may be more appropriate.

For example, you may want to eliminate the absorbance of a reagent solution from the test result. The Reader can hold the OD value of this reagent solution in memory and subtract it from all subsequent OD readings. Refer to the Blanking section in Chapter 2.

The appearance of the Blanking menu depends on which wells you chose when you defined the template.

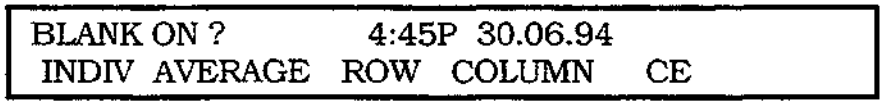
1. If your template contains no Blank wells, the Reader will offer you: air, last or plate blanking.



2. If your template contains a total number of Blank wells equal to the sum of the Standard, Test and Control wells, the Reader will offer you: paired blanking.

If you choose **NO** the third Blank menu is displayed.

3. If your template contains from 1 to 95 blanks, the Reader will offer you: average, individual, row or column blanking.



4. If 96 blanks were specified, whole blank mode is automatically set.

These blanking modes are described in the sections below.

Air Blanking

The Reader uses the absorbance of air as a reference level for 100% transmission. This is the default blank mode. Blank wells do not need to be specified in the template when using this mode. If you choose this mode the display will move on to the Start mode menu.

Last Blanking

Last blanking lets you use the blank value from the previously read plate. If last mode is used after a test using whole mode, the whole of the previous plate is used as blank for the next. Refer to the discussion of Whole Blanking later in this section.

Plate Blanking

In plate blanking the Reader reads and processes a plate of ODs. It then holds these ODs in memory and when the plate is read again it subtracts the previous set of 96 readings. This means that you can measure a change in sample concentrations over a period of time without monopolizing the Reader. Two tests are needed:

1. Create an assay test procedure for the first reading which uses air blanking. The data from this plate will be stored with an ID.
2. When the first test has been run, create a second test for the next reading. This test must use Plate blanking. The Reader will ask for the plate ID from the first test.

PLATE (00)	4:45P 30.06.94
DIR	ENTER CE

Scroll through the plates using the up and down cursor keys. When the correct plate is displayed select **ENTER**. Select **DIR** to print out a list of plates stored in memory.

Note: You may enter a plate number here which does not yet exist. For example, you may use the plate number which will correspond to the first of these two tests.

Individual Blanking

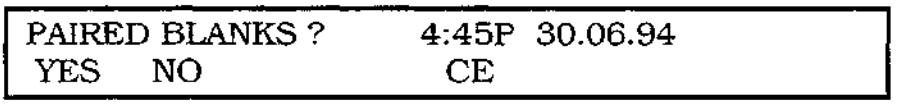
Individual blanking lets you position a series of Blank wells across a microplate. Each time the Reader encounters a Blank well, the blank value is updated. This value is then subtracted from subsequent ODs until the next Blank well is encountered. This is repeated until the whole plate has been read. Wells are read in the order A1 to H1, A2 to H2 etc.

Average Blanking

Average blanking averages the chosen series of wells into a single blank value, which is subtracted from each well OD on the plate.

Paired Blanking

In paired blanking, one blank is associated with each Test, Standard, or Control well. The blank may consist of one well or the average of a series of replicates. The total number of blank values must be equal to the total number of all the wells.



- **NO** If paired blanking is not selected, the next display offers the individual and average blanking menu.
- **YES** Blanks are assigned to different types of wells in the following order:

  Standards
  Tests
  Controls
  Positive Controls
  Negative Controls
  Cut-off Controls
  Positive Reference Controls

Example *For a plate with 3 S, 5 C and 8 T wells, 16 Blanks are required.*

Blanks 1 - 3 will be assigned to Standard wells S1 - S3.

Blanks 4 - 11 will be assigned to Test wells T1 - T8.

Blanks 12 - 16 will be assigned to Control wells C1 - C5.

Whole Blanking

Whole blanking mode should be used in conjunction with last mode. The Reader reads the plate and holds in memory an average of all 96 ODs. If last mode is selected for the next test this average blank value is subtracted from each OD on the next plate.

Row Blanking

In row blanking, Blanks may be specified within each row. The blank value is used within that row only. Each row is treated as separate from the others, with its own set of blanks.

Column Blanking

In column blanking, Blanks may be specified within each column. The blank value is used within that column only. Each column is treated as separate from the others, with its own set of blanks.

Results Matrix

A results matrix is a table of values, printed out when the OD data is processed. The Reader asks if you want to print a matrix of ODs.

```
O.D. MATRIX ?          4:45P 30.06.94
 NO    YES   BOXED LABELLED   CE
```

- **YES** Includes an OD matrix in the results.
- **BOXED** Includes a matrix with curve fit, threshold, difference, or ratio sections, if these have been specified.
- **LABELLED** Includes a matrix which shows the label of each well, for example S1, T5, C2 with curve fit, threshold, difference, or ratio sections if these have been specified.

Area Statistics

The Reader asks if you want to include area statistics in the results. You can divide the template into up to 50 sections (numbered 0 – 49) and for each of these sections the Reader will calculate the mean, standard deviation, standard error mean and coefficient of variation. These will then be output in the results. Areas can range from a single well to the complete microplate. Each area is defined by labelling the well locations involved with the number of the section. The Reader will then look for all the wells with the same number and view this as a discreet area.

```
AREA STATISTICS ?      4:45P 30.06.94
 YES   NO                 CE
```

Select **YES** to include area statistics and divide the template up into sections. Select **NO** to move on to the Quality Control equations.

```
A1 =                   4:45P 30.06.94
 ROW  COLUMN  CLEAR FINISH   CE
```

- **ROW** Places a row or part of a row within an area.
- **COLUMN** Places a column or part of a column within an area.
- **CLEAR** Removes the displayed well from its area. Clear can be used with **ROW** or **COLUMN** to remove rows and columns from areas.
- **FINISH** Ends the area statistics option. The Reader asks you to confirm that the template is correct. If necessary, you can print out the template using the **LIST** option to check that it is correct.

Placing Random Wells or Blocks of Wells in Areas

To place a random well in Area 0, scroll through the well locations using the cursor keys until you reach the required well. Enter the number 0 using the alphanumeric keypad; this well location is now assigned to Area 0. To place a block of wells in Area 0, scroll through the plate and place each well individually. Any other wells you label number 0 will also be included in Area 0.

Placing Rows in Areas

To place a row in Area 1, scroll through the well locations using the cursor keys until you reach the first well in the required row. Enter the number 1 using the alphanumeric keypad and select **ROW**. This row is now assigned to Area 1.

To place a portion of a row in Area 1, select the first well required, enter 1 and select **ROW**. Any other wells you label number 1 will also be included in Area 1.

Placing Columns in Areas

To place a column in Area 1, scroll through the well locations using the cursor keys until you reach the first well in the required column. Enter the number 1 using the alphanumeric keypad and select **COLUMN**. This column is now assigned to Area 1.

To place a portion of a column in Area 1, select the first well required, enter 1 and select **COLUMN**. Any other wells you label number 1 will also be included in Area 1.

Removing Rows and Columns from Areas

To remove an entire row from an area, scroll through the well locations using the cursor keys until you reach the first well of the required row. Select **CLEAR** and then **ROW**. To clear a portion of a row, select the first well used, select **CLEAR** and then **ROW**.

To remove an entire column from an area, scroll through the well locations using the cursor keys until you reach the first well of the required column. Select **CLEAR** and then **COLUMN**. To clear a portion of a column, select the first well used, select **CLEAR** and then **COLUMN**.

☞ *Note: If you move to a well which has been assigned an area number previously, you must use the **CLEAR** command to clear the previously assigned number before entering a new one.*

Quality Control Equations

You can include up to 12 Quality Control (QC) equations per assay. QC equations allow you to specify criteria for the OD data. If these criteria are not met, you may choose to abort the assay.

You can introduce conditions into the equation. That is, well ODs are accepted or rejected using criteria such as comparison with another specified well, or group of wells, or the use of a constant.

The Reader asks if you want to include a quality control equation.

```
QC1 EQN =_          4:45P 30.06.94
 WELL  OPTIONS  OPER   ENTER   CE
```

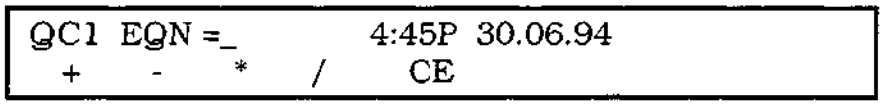
If you do not require quality control equations, select **ENTER** to move on to the output options section.

- **WELL** Inserts well type identifiers (Blank, Standard, Test, Control or User well) into the equation.
- **OPTIONS** Inserts brackets, inequality symbols, functions and statistical values into the equation.
- **OPER** Inserts arithmetic operators (+, -, *, /) into the equation.
- **ENTER** Saves the equation. The Reader checks the equation to test its validity. If it is not correct the Reader displays it for editing.
- **CE** Deletes the character above the cursor, or if no character has been added, it returns the display to the previous menu.

Use the keypad to enter numbers into the equation. Use the left and right cursor keys to scroll the equation left and right across the display. Use the up and down arrows to move to the start and end of the equation.

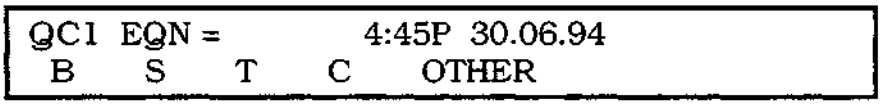
As you enter each quality control equation, the Reader increments to the next equation (for example from QC1 to QC2, etc). When no more equations are required, select **ENTER**.

OPER inserts an arithmetic operator into the equation



+	Addition	–	Subtraction
*	Multiplication	/	Division

WELL inserts a well type into the equation.



Select **B**, **S**, **T**, or **C** (Blank, Standard, Test or Control) to enter a well type, or select **OTHER** to access the User wells.

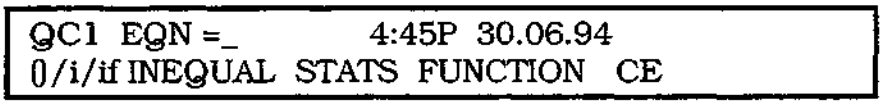
In QC equations, the well type identifier represents the mean OD of all the wells of that type. For example, **T** represents the average reading for all the Test wells on the plate. Using the keypad you can specify an individual sample using the sample number or an individual replicate using the sample and replicate numbers.

For example: C = the mean of all Control wells

C1 = the mean of all C1 replicates

C1.1= the OD of the first replicate of C1

OPTIONS inserts brackets, inequality symbols, functions and statistical values into the equation.



( )/i/if	Inserts brackets, an index counter (**i**) into the equation, or conditions (**if**, **then** and **else**).	
	( )	When inserting brackets, six levels of parentheses are allowed.
	i	Represents each defined well type. **Example 1:** Each Control (C) well OD must be within 25% of the mean of the all the Control wells, or that well fails quality control and is removed from the calculations. You have 4 Control wells (defined as C1, C2, C3, C4). The equation is written as: 0.75*C<Ci<1.25*C. **Example 2:** Referencing Example 1, this time you have two Control wells, each with a replicate of 3 (defined as C1.1, C1.2, C1.3 and C2.1, C2.2, C2.3). The equation is written as: 0.75*C<C1.i<1.25*C and 0.75*C<C2.i<1.25*C. If you need to have each replicate Control well OD fall within 25% of the mean of just the C1's or C2's, then the equation is written 0.75*C1<C1.i<1.25*C1 and 0.75*C2<C2.i<1.25*C2. Also, if you cannot accept any replicate wells above the Over Limit (see the *Microplate Reader User Manual*), the equation is written: C1.i<=4.0 or C2.i<=4.0. To fail the equation for any OVER readings in the replicate wells, use the Valid statement (for example, Valid (C1)>=3 and Valid (C2)>=3). Expressions containing **i**, often lead to ODs being excluded from the calculations. If every OD in a certain well type is excluded and this well type is used in another QC equation, the QC operation will not work properly. For this reason, each equation containing the **i** expression should be accompanied by a valid wells expression. This will ensure that if too many ODs are rejected the test will fail.

	if then else	Inserts a conditional expression into the equation. The Reader offers **if**, the first time this option is selected, **then** the second time, and **else** the third time. However, **else** is optional. **If** {condition} **then** {expression} **else** {expression} Example: **If** PC<0.2 **then** NC>0.5 **else** NC> 0.1. **If** the average of the PCs is less than 0.2, **then** the average of the NCs must be more than 0.5, or **else** the average of the NCs may be less than 0.1.
INEQUAL	Inserts inequality symbols (>, >=, <, <=) into the equations.	
STATS	Inserts a statistical value into the equation.	
	Median(	Represents the median of a group of ODs. If all ODs in the group are listed, the median is the OD in the middle of the list. If the list contains an even number of ODs, the median is the mean of the middle two ODs. The group of wells may be a well type, or a sample if it contains multiple replicates. Example: **Median(**PC) The value calculated will be the median of all the Positive Control ODs.
	SD(	Represents the standard deviation between the ODs in a group of wells. The group of wells may be a well type, or a sample if it contains multiple replicates.
	CV(	Represents the coefficient of variation between the ODs in a group of wells. The group of wells may be a well type, or a sample if it contains multiple replicates.

FUNCTION	Inserts a function into the equation.	
	Valid	Inserts a validity expression, that is, **valid**(well type identifier) {expression} Example: valid(PC)>=3 The number of valid PC wells (that is, those which pass the quality control equations) must be greater than or equal to 3, or the test fails quality control.
	Max/Min	Represents the maximum/minimum OD on the plate, or within a group of wells. The group of wells may be a well type, or a sample if it contains multiple replicates. Example 1: **Min**(T1) The lowest OD of all replicates of Test sample 1. Example 2: **Min** The lowest OD on the plate.
	Variable	Represents a variable which must be entered at runtime. Five variables are allowed per test. Example: **variable1** You will have to enter this number at runtime.

QC Equation Components

A summary of the components which can be used in quality control equations is shown below, together with a route showing you where to find them from the QC Equation menu.

Item	Example	Where found:
Numbers	**0-9**	Alphanumeric Keypad
Arithmetic Operators	**/ * – +**	Select **OPER**
To the power of	**	Select **OPER**
Brackets	**( )**	Select **OPTIONS, ( )/i/if**
Inequality Symbols	**> >= < <=**	Select **OPTIONS, INEQUAL**
Median of a list of ODs	**median(T)**	Select **OPTIONS, STATS**
Standard Deviation	**SD(PC1)**	Select **OPTIONS, STATS**
Coefficient of Variation	**CV(T)**	Select **OPTIONS, STATS**
Conditions	**if..then..else**	Select **OPTIONS, ( )/i/if**
Valid Wells	**valid(T)**	Select **OPTIONS, FUNCTION**
Each well of a well type: Well type and i	**Ti, NCi, PCi,**	Select **OPTIONS, ( )/i/if**
Well ODs: Well type and well number	**T1, S2, PC3**	Select **WELL**. Select the well type and enter the number on the alphanumeric keypad
Replicate ODs: Well type, number and replicate number.	**T1.2, B2.1**	Select **WELL**. Select the well type and enter the numbers on the alphanumeric keypad
Average ODs: Well type	**T, NC, PC**	Select **WELL**
Minimum OD	**Min(T)**	Select **OPTIONS, FUNCTION**
Maximum OD	**Max(S1)**	Select **OPTIONS, FUNCTION**
Variable	**variable1-5**	Select **OPTIONS, FUNCTION**

Examples of QC equations are shown below.

Example 1

Template includes: 3 Negative Controls and 2 Positive Controls:

If any Negative Control OD is more than 25% higher or lower than the mean of all the Negative Controls it must be rejected:
For **QC1** enter **0.75*NC<=NCi<=1.25*NC**

If more than one Negative Control OD is rejected the test fails QC:
For **QC2** enter **valid(NC)>=2**

The average of the Negative Control ODs must be more than 0.5:
For **QC3** enter **NC>0.5**

The average of the Positive Control ODs must be less than 20% of the average of the Negative Control ODs:
For **QC4** enter **PC<0.2*NC**

Example 2 *Template includes: 4 Negative Controls and 2 Positive Controls*

If any Negative Control OD is more than three times higher than the sum of the other three it must be rejected:
For **QC1** enter **4*NC-NCi>=NCi**

If more than two Negative Control ODs are rejected the test fails QC:
For **QC2** enter **valid(NC)>=2**

The average of the Negative Control ODs should not be greater than 0.2. However, if the average of the Positive Control ODs is greater than 0.7, then average Negative Control ODs of up to 0.3 will be accepted:
For **QC3** enter **If PC>0.7 then NC<=0.3 elseNC<=0.2**

The average of the Positive Control ODs must be more than 0.5:
For **QC4** enter **PC>0.5**

Inserting Output Options Into the Test

The output options command gives you a choice of various data processing options

```
SELECT OPTION          4:45P  30.06.94
THRESH  RATIO  CURVE  DIFF    EXIT
```

- **THRESH** Inserts a threshold matrix into the test, which lets you define the ranges for positive and negative results, and to perform quality control checks on the Control well ODs.
- **RATIO** Inserts a ratio matrix into the test, which lets you transform the ODs of Control and Test wells into different unit measurements.
- **CURVE** Inserts a curve fit section into the test, which lets you plot a graph of Standard ODs against concentrations.
- **DIFF** Inserts a difference matrix into the test, which lets you calculate the difference between ODs from two sets of wells. For example, the top and bottom halves of a microplate.
- **EXIT** Results are output in the form of a results matrix only. The test is saved and the Reader asks if you want to protect the test with a password.

Password

When you select **EXIT** to save a test, the Reader lets you protect it with a password to prevent unauthorized changes.

PASSWORD PROTECT ?	4:45P 30.06.94
YES NO	CE

Select **YES** to enter a password. Passwords may be up to 12 characters in length and consist of the following characters:

- Numbers 0–9 and a decimal point.
  These are entered using the alphanumeric keypad.
- Upper-case letters, lower-case letters and symbols.
  Enter these as follows:

  a) Choose upper-, or lower-case letters or the symbols using the up and down direction keys (∧ ∨)

  a) Scroll through the characters on the display with the left and right direction keys (< >). Enter the character shown by the flashing cursor by pressing the (∧) selection key.

The Reader will ask you to enter the password twice to make sure that it is correct. When you have entered the password twice, select **ENTER**. The test will be saved and may only be edited, copied, or overwritten if the correct password is entered.

Note: The password protection option should be used with caution; if you forget the password, you will not be able to edit or overwrite the test. Contact an approved service centre for advice.

Threshold Matrix Output Option

The threshold matrix is printed as a table of symbols, denoting OD data. The threshold matrix section lets you define ranges for positive and negative ODs. If there is a large neutral area between positive and negative, you may define segments within this area. If the positive cut-off value is equal to the negative cut-off value, you can designate an assay as "Inverse." You can also use quality control (QC) equations to specify which Controls will be used and which will be rejected.

SELECT OPTION	4:45P 30.06.94
THRESH RATIO CURVE DIFF EXIT	

To include a threshold matrix, select **THRESH**. The Reader asks if you only require QC equations.

```
Q.C. ONLY ?          4:45P 30.06.94
 YES    NO                    CE
```

Select NO to specify threshold limits, before inserting QC equations. Select YES to insert QC equations and omit threshold limits.

Defining the Threshold Ranges

If you choose to use threshold ranges as well as QC equations, the Reader will ask you to enter the limits for the positive and negative ranges. These ranges are defined by threshold equations.

The Reader will ask you to enter the negative equation (which defines the negative range) and then the positive equation (which defines the positive range). A positive and negative range will always be required, but two extra positive levels (++ and +++) are also available. These can be used to show how positive each result is.

Threshold limits can be entered directly as absolute numbers (that is, a result will be recorded as positive or negative if it falls above or below a specified number).

It may be necessary to introduce conditions into the equation (that is, a result will be recorded as positive or negative using certain other criteria, such as comparison with a specified well, or group of wells, or the use of a constant).

Threshold Equations

The Reader asks you to enter the negative equation, to define the negative range.

```
 - EQN =_            4:45P 30.06.94
WELL  OPTIONS OPER  ENTER   CE
```

WELL Inserts well type identifiers (Blank, Standard, Test, Control or User well) into the equation.

OPTIONS Inserts brackets and statistical values into the equation.

OPER Inserts arithmetic operators (+, -, *, /) into the equation.

*Note: Two * characters together indicate 'to the power of', e.g,. T1**PC*

ENTER Saves the equation. The Reader checks the equation to test its validity. If it is not correct, the Reader displays it for editing.

CE Deletes the character above the cursor, or if no character has been added, it returns the display to the previous menu.

Use the keypad to enter numbers into the equation. Use the left and right cursor keys to scroll the equation left and right across the display. Use the up and down arrows to move to the start and end of the equation.

As you enter each threshold equation, the Reader increments to the next equation (for example from the positive equation to the double positive equation, etc.). If you do not want to use one of the equations, select **ENTER**.

The value which results from the - equation is called the - value. The value which results from the + equation is called the + value.

If the - and + equations are identical:

for example: + EQN = NC + .250

- EQN = NC + .250

then: ODs below the - value are negative

ODs equal to or above the + value are positive

If the - equation is higher than the + equation, as in an inverse assay:

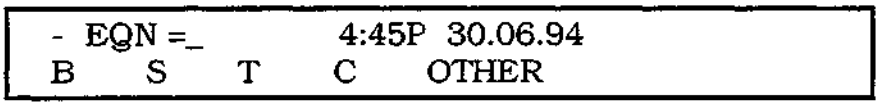
for example: + EQN = 0.2 * (NC + PC) - 0.001

- EQN = 0.2 * (NC + PC)

then: ODs below or equal to the + value are positive

ODs above the - value are negative

WELL inserts a well type identifier (Blank, Standard, Test, Control, or User well).



Select **B**, **S**, **T**, or **C** (Blank, Standard, Test or Control) to enter a well type, or select **OTHER** to access a second menu. If you have changed the name of any user wells (refer to the User Wells section in Chapter 4) the appropriate label will be displayed.

In Threshold equations, the well type identifier represents the mean OD of all the wells of that type. For example, **T** represents the average reading for all the Test wells on the plate.

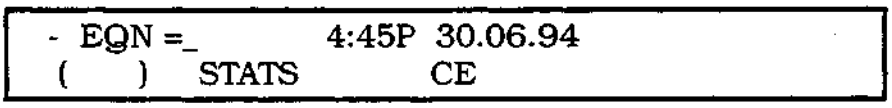
Using the keypad you can specify an individual sample using the sample number or an individual replicate using the sample and replicate numbers.

For example: C = the mean of all Control wells

C1 = the mean of all C1 replicates

C1.1= the OD of the first replicate of C1

OPTIONS inserts brackets and statistical values into the equation.



( )	Inserts left and right brackets into the equation. Six levels of parentheses are allowed.	
STATS	Inserts a statistical value into the equation.	
	Median(	Represents the median of a group of ODs. If all ODs in the group are listed, the median is the OD in the middle of the list. If the list contains an even number of ODs, the median is the mean of the middle two ODs. The group of wells may be a well type, or a sample if it contains multiple replicates. Example: **Median**(PC) The value calculated will be the median of all the Positive Control ODs.
	SD(	Represents the standard deviation between the ODs in a group of wells. The group of wells may be a well type, or a sample if it contains multiple replicates.
	CV(	Represents the coefficient of variation between the ODs in a group of wells. The group of wells may be a well type, or a sample if it contains multiple replicates.

OPER inserts an arithmetic operator into the equation

+	Addition	–	Subtraction
*	Multiplication	/	Division

Threshold Equation Components

A summary of the components which can be used in threshold equations is shown below, together with a route showing you where to find them from the Threshold Matrix menu.

Item	Example	Where found
Numbers	**0-9**	Alphanumeric Keypad
Arithmetic Operators	**/ * - +**	Select **OPER**
To the power of	**	Select **OPER**
Brackets	**( )**	Select **OPTIONS**
Median of a list of ODs	**median(T)**	Select **OPTIONS, STATS**
Standard Deviation	**SD(PC1)**	Select **OPTIONS, STATS**
Coefficient of Variation	**CV(T)**	Select **OPTIONS, STATS**
Well ODs: Well type and well number	**T1, NC2, PC3, CO2**	Select **WELL**. Select the well type and enter the number on the alphanumeric keypad
Replicate ODs: Well type, number and replicate number.	**T1.2, S2.1, PC3.2**	Select **WELL**. Select the well type and enter the numbers on the alphanumeric keypad
Average ODs: Well type	**T, CO, S**	Select **WELL**

Example 1

Positive — ODs above 1.0000 must be recorded as positive.

For the **+ positive** equation enter: **1.0000**

Negative — ODs below 0.9999 must be recorded as negative.

For the **– negative** equation enter: **0.9999**

Example 2

Positive — ODs above the result of Cut off Control 1 plus 0.4 must be recorded as positive.

For the **+ positive** equation enter: **CO1+0.4**

++ Positive — ODs above the result of Cut off Control 1 plus 0.6 must be recorded as double positive.

For the **++ positive** equation enter: **CO1+0.6**

+++ Positive — ODs above the result of Cut off Control 1 plus 0.8 must be recorded as triple positive.

For the **+++ positive** equation enter: **CO1+0.8**

Negative — ODs below the result of Cut-off Control 1 minus 0.4 must be recorded as negative.

For the **– negative** equation enter: **CO1– 0.4**

Segments

If there is a large difference between the lower limit of the positive range and the upper limit of the negative range, many readings may fall between the two. This neutral area may be divided into as many as ten segments which are numbered 0 to 9.

For example, if there are two segments and the OD for well A1 falls into the second segment, it will be recorded in the matrix by the number 1.

```
NO. OF SEGMENTS =      4:45P  30.06.94
                    ENTER   CE
```

To define the number of segments other than one, enter the number using the alphanumeric keypad.

☞ *Notes:*

1. *When doing an inverse assay and the positive and negative equations are the same, there is no neutral area; therefore, this prompt will not appear.*
2. *The segments are numbered upwards from the negative end of the neutral area. So that, the highest number is the closest to a positive result.*

Labels

The Labels option lets you choose how the positive and negative results will appear in the printed threshold matrix. The default labels are – for negative, + for positive, ++ for double positive, +++ for triple positive and 0 for the neutral area (if only one segment has been defined).

The Reader displays the current label. Select **ENTER** to confirm this label, or **CE** to change it, and access the letters and symbols. For example:

```
- LABEL =              4:45P  30.06.94
   YZ ABCD              ENTER   CE
```

Use the up and down cursor keys to access the lower-case letters and symbols and the left and right cursor keys to scroll through the characters. Press the function key to enter a character or **CE** to delete a character. Use the alphanumeric keypad to enter numbers. Labels may be up to five characters in length. When the label is complete, select **ENTER** to accept it.

The Reader also assigns default labels to other types of data, such as unread wells and out of range readings. These labels cannot be changed:

*****	Unread well.
*	Out of range data.
#	Combined data. This symbol denotes difference data. It is found in the higher level of wells which have been used in difference calculations. Difference data will only be included in a threshold matrix if a difference matrix was placed before the threshold matrix.

QC Equations

When you have defined the labels or if **YES** was selected at the **Q.C. ONLY** prompt, the Reader asks you to enter QC equations.

You can include up to 12 Quality Control (QC) equations per assay. QC equations allow you to specify criteria for the **calculated results**. If these criteria are not met, you may choose to abort the assay.

The threshold quality control equations are entered in the same way as the ordinary QC equations. Refer to the Quality Control Equations section earlier in this chapter.

When you have completed the threshold matrix section, the display returns to the Output Options menu. If you want to end the test, select **EXIT**. The Reader will ask if you want to protect the test with a password. Refer to the Password section earlier in this chapter.

Threshold Limits

The threshold matrix will determine if your samples are Negative (-), Positive (+), Double Positive (++), Triple Positive (+++) or if they fall between the Negative and Positive (Equivocal). The threshold limits are determined by entering either a specific number (OD value) or an equation (floating cut-off) using well types and options.

The Reader prompts you as follows:

- **-EQN=** Everything less than this entry will be labeled or denoted as negative.
- **+EQN=** Everything greater than or equal to this entry yet less than the ++ value will be labeled or denoted as + positive.
- **++EQN=** Everything greater than or equal to this entry yet less than the +++ value will be labeled or denoted as ++ positive.
- **+++EQN=** Everything greater than or equal to this entry will be labeled or denoted as +++ positive.

Example 1

OD's that are less than 0.200 are negative and OD's greater than or equal to 0.200 and less than 1.000 are a + positive and OD's greater than or equal to 1.000 show a strong positive.

-EQN= 0.1999

+EQN= 0.2000

++EQN= 1.000

Example 2

Test samples are negative if they are lower than the Negative Control well and are positive if they equal or are greater than the Positive Control well.

-EQN= NC-.0001

+EQN= PC

Inverse Assays

If you enter equal positive and negative cut-off values, the Reader asks if you want to designate the assay as inverse. If you specify inverse assay, a well is marked as positive (+) if the result of the threshold equation is less than or equal to the positive cut-off value. A well is marked as negative (-) if the result of the threshold equation is greater than the negative cut-off.

```
INVERSE ASSAY ?          4:45P 30.06.94
 YES   NO                CE
```

Select **YES** to designate the assay as inverse. Select **NO** for a standard assay.

Ratio Matrix Output Option

The ratio matrix is a table of results obtained by converting the ODs into a different scale than they were read in. This is accomplished by multiplying all the ODs on the plate by a ratio equation. The ratio equation is calculated once for every well on the plate and the result replaces the OD value, for subsequent calculations.

You can also use an International Units (IU) equation to convert the results to different units, which will appear in the ratio matrix as an extra set of IU conversions. A typical ratio matrix is shown in Appendix B.

To include a ratio matrix select **RATIO** at the output options prompt. The Reader asks you to enter the ratio equation.

```
RATIO = _          4:45P 30.06.94
WELL  OPTIONS OPER   ENTER   CE
```

- **WELL** Inserts well type identifiers (Blank, Standard, Test, Control or User well) into the equation.
- **OPTIONS** Inserts brackets, functions and logarithmic functions into the equation.
- **OPER** Inserts arithmetic operators (+, -, *, /) into the equation.
- **ENTER** Saves the equation. The Reader checks the equation to test its validity. If it is not correct the Reader displays it for editing.

- CE — Deletes the character above the cursor, or if no character has been added, it returns the display to the previous menu.

Use the keypad to enter numbers into the equation. Use the left and right cursor keys to scroll the equation left and right across the display. Use the up and down arrows to move to the start and end of the equation.

When you have entered the ratio equation the Reader moves on to the IU equation. If you do not want to enter an IU equation or after you have finished the IU equation, select **ENTER**.

Ratio and IU equations may be up to 50 characters in length and may be used to scale the data up or down.

WELL inserts a well type into the equation.

```
RATIO EQN =          4:45P 30.06.94
 B     S     T     C     OTHER
```

Select **B**, **S**, **T**, or **C** (Blank, Standard, Test or Control) to enter a well type, or select **OTHER** to access the User wells.

In RATIO equations, the well type identifier represents the mean OD of all the wells of that type. For example, **T** represents the average reading for all the Test wells on the plate. Using the keypad you can specify an individual sample using the sample number or an individual replicate using the sample and replicate numbers.

For example: C = the mean of all Control wells

C1 = the mean of all C1 replicates

C1.1= the OD of the first replicate of C1.

OPTIONS inserts brackets, mathematic functions and logarithmic functions into the equation.

```
RATIO = _          4:45P 30.06.94
 (    ) FUNCTION MATH     CE
```

( )	Inserts left and right brackets into the equation. Six levels of parentheses are allowed.	
FUNCTION	Inserts a mathematical function into the equation.	
	Sample	inserts the individual OD of every well on the plate into the equation.
	Variable	represents a variable which must be entered at runtime. Five variables are allowed per test. Example: **variable1** You will have to enter this number at runtime.
	Max/Min	represents the maximum/minimum OD on the plate, or within a group of wells. The group of wells may be a well type, or a sample if it contains multiple replicates. Example 1: **Min**(T1) The lowest OD of all replicates of Test sample 1. Example 2: **Min** The lowest OD on the plate.
MATH	Inserts a logarithmic function into the equation.	
	Log	inserts a base 10 logarithm
	Alog	inserts an inverse base 10 logarithm
	Ln	inserts a natural logarithm
	Exp	inserts an inverse natural logarithm

OPER inserts an arithmetic operator into the equation.

+	Addition	-	Subtraction
*	Multiplication	/	Division

Ratio and IU Equation Components

A summary of the components which can be used in ratio and IU equations is shown below, together with a route showing you where to find them from the Ratio Matrix menu.

Item	Example	Where found
Numbers	**0-9**	Alphanumeric Keypad
Arithmetic Operators	**/ * - +**	Select **OPER**
To the power of	**	Select **OPER**
Brackets	**( )**	Select **OPTION**
Logarithmic Functions	**log alog ln exp**	Select **OPTION, MATH**
1 to 5 variables	**for example variable1**	Select **OPTION, FUNCTION**
Minimum OD	**Min(T)**	Select **OPTION, FUNCTION**
Maximum OD	**Max(S1)**	Select **OPTION, FUNCTION**
Maximum plate OD	**max**	Select **OPTION, FUNCTION**
Individual OD of every well	**sample**	Select **OPTION, FUNCTION**
Individual replicate ODs	**for example sample1**	Select **OPTION, FUNCTION** and enter the number on the alphanumeric keypad
Well ODs: Well type and well **number**	**T1, NC2, PC3, CO2**	Select **WELL**. Select the well type and enter the number on the alphanumeric keypad
Replicate ODs: Well type, number and replicate number.	**T1.2, NC2.3, PC4.2, S2.1**	Select **WELL**. Select the well type and enter the numbers on the alphanumeric keypad
Average ODs: Well type	**T, NC, PC, C, CO, S**	Select **WELL**

Example

To convert all ODs on the plate to a ratio of the average Positive Control. Then to convert into different units (15 times smaller):
For the **Ratio** equation enter: **sample/PC**
For the **IU** equation enter: **sample/15**

When you have completed the ratio matrix section, the display returns to the Output Options menu. If you want to end the test, select **EXIT**. The Reader will ask if you want to protect the test with a password. Refer to the Password earlier in this chapter.

Curve Fitting Output Option

The curve fitting routines can be used to interpret data. ODs are quantified by comparing them to a concentration graph which is constructed from the concentrations and ODs of Standards. The curve fit section lets you specify which type of graph is used, and other conditions about how the data is to be treated.

```
SELECT OPTION          4:45P  30.06.94
THRESH  RATIO  CURVE  DIFF   EXIT
```

To include a curve fit select **CURVE**.

Several stages are involved in defining a curve fit:

Option	Description
Results Format	Select the results format for the curve (concentration or titer)
Use Stored Curve	Use a previously defined and stored curve
Reagent Concentration	Enter the reagent concentrations used in the Standard wells (if Standards are defined in the template)
Curve Fit Type	Select a curve fit type (if Standards are defined in the template)
Slope	Enter the slope and the y axis intercept of the curve (if Standards are not defined in the template)
Axis fit	Select the type of scale to be used for the x and y axes of the printed graph
Units	Enter the concentration units used in the test
Use Tails	Extrapolate the curve beyond the range defined by the data points.
Sample Dilutions	Apply dilution factors to test, control and user wells
Curve Fit Quality Control Limits	Apply a series of limits to the curve fit data
IU Equation	Apply an IU equation

Results Format

The Reader asks for the results format.

```
RESULT FORMAT ?        4:45P 30.06.94
 CONC.  TITER                  CE
```

CONC. The Reader will ask you to enter Standard concentrations in concentration values

TITER The Reader will ask you to enter Standard concentrations in titer values.

Stored Curve

The Reader asks if you want to use a stored curve.

```
USE STORED CURVE ?     4:45P 30.06.94
 YES    NO                    CE
```

The stored curve function lets you apply the parameters of a previously defined curve to new data. Select **YES** to specify use of a stored curve. The Reader will ask for the number under which the curve is stored.

```
CURVE (00)             4:45P 30.06.94
                DIR   ENTER   CE
```

Enter the number of the stored curve and select **ENTER**. Select **DIR** to print a list of all the stored curves.

☞ *Note: If you have defined Standards in the template, the Reader will ask you to enter the concentration of the Standards. If you have not defined Standards in the template, the Reader will ask you to enter the slope of the curve manually, refer to the Slope and Y Axis Intercept section later in this chapter.*

Reagent Concentration

The Reader asks you to enter the concentrations of the Standards. You will either have to enter a concentration value or a titer value, depending on which was chosen in the Results Format menu.

```
CONC. OF S1 =          4:45P 30.06.94
       e              ENTER   CE
```

For Sigmoid (4PLC), semi-log and log-log fits –
Minimum: 0.001, maximum 1e200 ($1x10^{200}$)

For all other fits –
Minimum: 0.0, maximum 1e200 ($1x10^{200}$).

```
TITER OF S1 = 1:        4:45P  30.06.94
        e              ENTER   CE
```

Minimum: 1, maximum 999999.

Specify the concentration and select **ENTER**. Numbers are entered using the alphanumeric keypad, the exponential notation **e** is entered by pressing the ^ key below it.

Curve Fit Type

The Reader will ask you which type of curve fit you require.

```
CURVE FIT TYPE ?        4:45P  30.06.94
 POWER  SPLINE  POLYGON  OTHER     CE
```

Select **POWER**, **SPLINE**, or **POLYGON** to specify a fit type, or select **OTHER** to access a second menu.

```
CURVE FIT TYPE ?        4:45P  30.06.94
SIGMOID MICHAEL  AKIMA            CE
```

- **POWER** Polynomial regression routine: linear regression, quadratic equation, cubic equation or quartic equation are available
- **SPLINE** Cubic spline fit
- **POLYGON** Successive points are joined by straight lines
- **SIGMOID (4PLC)** Sigmoidal S-shaped fit
- **MICHAEL** Michaelis-Menten hyperbolic fit
- **AKIMA** Curve which passes through every point and appears smooth and natural, much like a manually drawn curve.

In the case of Spline, Polygon, Akima and Power modes data is transformed before curve fitting is performed. Refer to Appendix C. For Sigmoid (4PLC) and Michaelis-Menten functions, the best fit is calculated using untransformed data.

Select **SIGMOID** to define any of the four parameters of the sigmoid (4PLC) curve.

```
FIX PARAMETERS ?        4:45P  30.06.94
 LOGIT   YES    NO              CE
```

- **LOGIT** lets you fix just the A and D parameters.
- **YES** lets you fix all four parameters.

Select **LOGIT** or **YES** to alter parameters. A value for the relevant parameter will be displayed. Select **ENTER** to accept this value or **CE** to change it. Numbers are entered using the alphanumeric keypad, the exponential notation **e** and the **+/-** signs are entered by pressing the ^ key below them.

Parallel Line Bioassays

Parallel line bioassays are used to calculate relative dosages between a set of up to 12 curves. A set of Standards defines the standard curve and sets of Test wells of identical size define the additional test curves. If the test and standard curves are parallel, the test data is valid. Dosages can be determined from the position of the test curves relative to the standard curve.

The following conditions must be met for a parallel line bioassay to be available as a curve fit option:

- **Standard curve** There must be at least two Standards and the Standards must be in double, triple or quadruple replicates.
- **Test Curve** Test wells must be arranged in the same number of replicates as the Standard wells.
- **Sets of Test curves** The number of Test wells must be a multiple of the number of Standards. For example, a test sequence with eight Standards can have 8, 16, 24, etc. Test wells.

If these three conditions have been met, the **PARALLEL** option can be selected from the **OTHER** Curve Fit Types menu.

```
CURVE FIT TYPE ?      4:45P 30.06.94
SIGMOID MICHAEL AKIMA PARALLEL   CE
```

Parallel

The statistical technique Analysis of Variance (ANOVA) is used to determine if the test data for the parallel line bioassay is valid. All curves must be parallel for the test to be valid. If not, the worst cases are rejected until the remaining curves are parallel or all the data has been rejected.

Select **PARALLEL** to specify a parallel line bioassay. The Reader will ask you to enter the units for the curve.

Use the left and right cursor keys to scroll through the characters. Press the function key to enter a character or **CE** to delete a character. Use the alphanumeric keypad to enter numbers. When the units label is complete, select **ENTER** to accept it.

If more than 12 curves are entered, or all of the test curves are rejected then a **PARALLEL LINE ERROR** message will be displayed and the calculations will be aborted.

☞ *Note: Parallel line bioassay data cannot be passed to a threshold matrix.*

Slope and Y Axis Intercept

If you have not defined Standards in the template only linear regression is available. You will have to input the slope of the line and the Y axis intercept point manually.

```
SLOPE =              4:45P  30.06.94
      e     +/-    ENTER   CE
```

Minimum: +/-0.0001, maximum: 1e200.

Enter the value for the slope using the keypad and select **ENTER**. The Reader will ask for the Y axis intercept point.

```
Y-INT =              4:45P  30.06.94
      e     +/-    ENTER   CE
```

Minimum: +/-0.000, maximum: 1e200.

Enter the value for the slope using the keypad and select **ENTER**.

Axis Fit

The Reader asks for the type of axis fit required. This option lets you choose how data is fitted onto the axes of the printed graph.

```
AXIS FIT ?           4:45P  30.06.94
LINEAR SEMI-LOG LOG-LOG  AUTO      CE
```

- **LINEAR** Linear scales for both axes.
- **SEMI-LOG** Uses a logarithmic scale for the x axis.
- **LOG-LOG** Uses a logarithmic scale for both axes.
- **AUTO** The Reader selects the best axis fit automatically (only available for the linear regression, quadratic, cubic and quartic fits).

☞ *Note: For a Sigmoid (4PLC) curve fit, you should use a Semi-Log axis fit.*

Tails

The curve shown on the printout can be extrapolated beyond the values defined by the test data by selecting the tails option.

```
USE TAILS ?          4:45P 30.06.94
 YES    NO                 CE
```

This option should be used cautiously particularly with the higher order polynomial (for example quartic) or spline curve fits. Refer to Appendix C.

Units

The Reader asks for the concentration units which were used for the test. These units will be printed out on the x axis of the graph.

```
UNITS =              4:45P 30.06.94
     YZ ABCD          ENTER   CE
```

Use the up and down cursor keys to access the lower-case letters and symbols and the left and right cursor keys to scroll through the characters.

Press the function key to enter a character or **CE** to delete a character. Use the alphanumeric keypad to enter numbers.

When the units label is complete, select **ENTER** to accept it.

Curve Fit Quality Control Limits

Quality control limits can be applied to the curve fit data. If the curve plotted from the Standards data does not meet the requirements of the quality control expressions, a **FAILED** message is printed.

```
CURVE FIT Q.C. ?     4:45P 30.06.94
 YES    NO                 CE
```

Select **YES** to define values for the limits listed below:

Limit	Description
MIN R-SQR	The minimum R^2 value.
MIN Y-INT	The minimum intercept of the curve fit plot through the y-axis (only for linear regression, cubic, quartic and quadratic fits).
MAX Y-INT	The maximum intercept of the curve fit plot through the y-axis (only for linear regression, cubic, quartic and quadratic fits).
MIN SLOPE	The minimum slope value for a linear fit
MAX SLOPE	The maximum slope value for a linear fit

Enter the values and select **ENTER**.

Scale Graph

The **SCALE GRAPH** option lets you scale the size of the graph up and down. If you do not specify a range here, the Reader will automatically scale the graph to the standard range.

```
SCALE GRAPH?          4:45P 30.06.94
 YES    NO                    CE
```

Select **NO** to proceed to sample dilution. Select **YES** to scale the graph. The Reader asks for the maximum and minimum points of the x and y axes. For example:

```
X MINIMUM =           4:45P 30.06.94
        e     +/-   ENTER   CE
```

Numbers are entered using the alphanumeric keypad, the exponential notation e and the +/- signs are entered by pressing the ∧ key below them. When each point is correct, select **ENTER**. The Reader will increment to the next point (for example x minimum to y maximum, etc).

Sample Dilution

The Dilution option lets you apply a dilution factor to any of the Test, Control or User wells which have been diluted. The Reader displays well location A1 and its well type. For example:

```
A1 T1  1:1          4:45P 30.06.94
  ROW  COLUMN  CLEAR FINISH   CE
```

- **A1** well location - to move to a different well location scroll through the template using the cursor keys.
- **T1** well type and number (replicate number is not displayed.
- **1:1** dilution of the sample.
- **ROW** lets you apply the dilution factor you enter, to the whole row or part of the row.
- **COLUMN** lets you apply the dilution factor you enter, to the whole column or part of the column.
- **CLEAR** clears the currently displayed dilution allowing you to enter a new one.
- **FINISH** ends the dilution option.

If a Test, Control or User well has not been diluted enter 1:1. If any samples have been diluted, scroll through the template until you reach the well.

Select clear to delete the 1:1 ratio. The Reader will ask for the new dilution.

```
A1 T1  1:          4:45P 30.06.94
                 ENTER   CE
```

Enter the new dilution using the keypad. For a dilution of 1 volume of reagent to two volumes of diluent enter 1:2, and so on. When the dilution factor is correct, select **ENTER**. The Reader will return to the dilution prompt.

To assign the displayed dilution factor to the row you are in, select **ROW**. If you are at the start of a row, all the dilutions in that row will be overwritten and replaced with the displayed dilution factor. If you are not at the start of the row and you select **ROW**, the dilutions in the well locations after the displayed location, will be overwritten and replaced with the displayed dilution factor.

The **COLUMN** command works in the same way as the Row command, above.

International Units Equations

This option applies an International Units equation to the data.

```
I.U.  = _          4:45P 30.06.94
WELL  OPTIONS OPER   ENTER    CE
```

If you do not require an IU equation, select **ENTER**.

The curve fitting IU equation is entered in the same way as the ratio matrix IU equation. Refer to the Ratio Matrix section earlier in this chapter.

Results Table

The Reader asks if a table of Curve Fit results should be printed in the results output.

TABLE OF RESULTS	4:45P 30.06.94
YES NO	CE

Note: If the test already contains a number of processing options, the file may become very large and difficult to process if you also include a curve fit table. If the test crashes when you run it, try removing the curve fit table and running the test again.

When you have completed the curve fit section, the display returns to the Output Options menu. If you want to end the test, select **EXIT**. The Reader will ask if you want to protect the test with a password. Refer to the Password section earlier in this chapter.

Difference Matrix Output Option

The Difference Matrix is a table of data showing the differences in ODs between subsequent rows or columns of wells, or between one half of the microplate and the other.

SELECT OPTION	4:45P 30.06.94
THRESH RATIO CURVE DIFF EXIT	

To include a difference matrix select **DIFF**.

Note: If a difference matrix is inserted into the test, all subsequent output options will use the difference matrix results.

The Reader asks if the test is using an alternative format template.

ALT. FORMAT ?	4:45P 30.06.94
YES NO	CE

Select **YES** if you are using a non-standard format, that is, not a 8x12 format.

The Reader will ask whether you want to subtract halves of the plate or adjacent rows or columns.

SUBTRACT ?	4:45P 30.06.94
HALF ADJ.	CE

Half

HALF subtracts the reading in one half of the microplate from the readings in the other half of the plate.

The Reader will ask for the row/column orientation of the difference matrix.

SUBTRACT ?	4:45P 30.06.94
COLUMNS ROWS	CE

- **COLUMNS** Sets the microplate halves as columns 1 to 6 on one side and columns 7 to 12 on the other.
- **ROWS** Sets the microplate halves as rows A to D on one side and rows E to H on the other.

Adjacent

ADJ. subtracts the readings in adjacent rows or columns from each other.

The Reader will ask for the row/column orientation for the difference matrix.

SUBTRACT ?	4:45P 30.06.94
COLUMNS ROWS	CE

Select **COLUMNS** to subtract adjacent columns from one another, or **ROWS** to subtract adjacent rows from one another.

Subtraction Order

The order of the subtraction for rows, columns or halves must be specified.

SUBTRACT. ORDER ?	4:45P 30.06.94
1st-2nd 2nd-1st	CE

COLUMN and **1st - 2nd** calculates the following differences:

1 - 2, 3 - 4, 5 - 6, 7 - 8, 9 - 10, 11 - 12

COLUMN and **2nd - 1st** calculates the following differences:

2 - 1, 4 - 3, 6 - 5, 8 - 7, 10 - 9, 12 - 11

ROW and **1st - 2nd** calculates the following differences:

A - B, C - D, E - F, G - H

ROW and **2nd - 1st** calculates the following differences:

B - A, D - C, F - E, H - G

HALF and **1st - 2nd** calculates the following differences:

A1 - A7, B1 - B7.....H1 - H7, A2 - A8 – H6.....H12

HALF and **2nd - 1st** calculates the following differences:

A7 - A1, B7 - B7.....H7 - H1, A8 - A2.....H12 - H6

Note: Differences are always printed in the lowest well number of the operation. For example the result of E2–E1 will be printed in E1 and the result of B1–A1 will be printed in A1. The remaining wells (that is, in the examples above E2 and B1) will appear as #####.

When you have completed the difference matrix section, the display returns to the Output Options menu. If you want to end the test, select **EXIT**. The Reader will ask if you want to protect the test with a password. Refer to the Password section earlier in this chapter.

4.2 Editing a Test – EDIT

Select **EDIT** from the Program menu to edit an existing assay test procedure. The Reader will ask you for the number of the test you want to edit.

```
TEST (00)          4:45P 30.06.94
              DIR   ENTER   CE
```

Select **DIR** to print a list of the stored tests.

Use the keypad to type in a test number and then select **ENTER**. If you enter a number for which no corresponding test exists, the test number prompt will reappear. Re-enter a valid test number.

When a valid test number has been selected you must confirm it. For example:

```
NAME = RUBELLA      4:45P 30.06.94
 COPY         STOP   ENTER   CE
```

- **COPY** Copies the test to another test number.
- **STOP** Stops editing and saves the test.
- **ENTER** Confirms the test and moves to the next display.
- **CE** Cancels the test and lets you choose another.

Copy

Select **COPY** to make a copy of the test before editing the original. The display prompts you to give the copied test a new number.

```
TEST (00)          4:45P 30.06.94
              DIR   ENTER   CE
```

If you enter the number of an existing test, the Reader will ask if you want to choose another test, or overwrite this test. For example:

```
CLEAR TEST A10        4:45P 30.06.94
 YES   NO                  CE
```

- **YES** makes the copy, overwriting the old test of that number.
- **NO** cancels the copy instruction and returns to the **TEST NUMBER** prompt.

*Note: Do not use the original test number for the copied test. If you do, the original test will be lost when you select **CLEAR TEST**, and it will no longer exist to be copied. If this occurs an **INVALID COPY** message is displayed.*

Editing Tests Options

When a valid number has been entered you must confirm it, then you may begin editing the test. The Reader displays each step of the test and lets you confirm the step or edit it. The first step displayed will be the plate ID prompt.

```
ID PROMPT : YES       4:45P 30.06.94
            STOP   ENTER   CE
```

At any time during editing

- **STOP** Saves the test and returns to the Program menu.
- **ENTER** Accepts the displayed test step and moves on through the test.
- **CE** Lets you alter the displayed test step.

You can move through the test, step by step, by selecting **ENTER** at each step. In this way all the steps in a test can be viewed.

To change any of the test steps, select **CE** to access the alternatives.

Editing the Template

You may edit the template by scrolling through the test steps to the **TEMPLATE** section:

```
TEMPLATE CORRECT ?    4:45P 30.06.94
 YES   LIST                CE
```

Select **YES** to continue to the next step in the test or **LIST** to print out the template. Select **CE** to view the template well by well and redefine it if necessary.

Editing the Output Options

When the **THRESH**, **RATIO**, **CURVE** or **DIFF** options are displayed, several additional editing tools become available. For example:

```
DIFFERENCE OPTION      4:45P  30.06.94
INCLUDE DELETE  STOP   EDIT   INSERT
```

- **INCLUDE** Includes the named option as previously defined and displays the next option in the test.
- **DELETE** Deletes the displayed option.
- **STOP** Saves the test and returns to the Program menu.
- **EDIT** Lets you modify the displayed option.
- **INSERT** Inserts an additional option from the Output Options menu. Refer to the Inserting Output Options Into the Test section earlier in this chapter.

No Option If there are no output options in the test, if they have all been deleted or if **INCLUDE** has been incremented past the last option, the following message will be displayed:

```
NO OPTION              4:45P  30.06.94
            STOP          INSERT
```

Select **STOP** to save the test or **INSERT** to insert a **THRESH**, **RATIO**, **CURVE** or **DIFF** option.

4.3 Printing Tests – LIST

Select **LIST** from the Program menu to print a test on a connected printer. Select the number of the test you want to print.

```
TEST  (00)             4:45P  30.06.94
            DIR   ENTER   CE
```

Select **DIR** to print a list of the stored tests.

Use the keypad to type in a test number and then select **ENTER**. If you enter a number for which no corresponding test exists, the test number prompt will reappear. Re-enter a valid test number.

When a valid test number has been selected you must confirm it. For example:

```
TEST  (10) RUBELLA     4:45P  30.06.94
            DIR   ENTER   CE
```

Select **ENTER** to confirm the test; the test will be printed.

4.4 Printing Directories – DIR

Select **DIR** from the Program menu to print the directory of:

- **Up to** 100 stored assay test procedures
- **Up to** 100 plate IDs
- **Up to** 20 stored curves

```
DIRECTORY OF          4:45P  30.06.94
 TESTS  PLATES  CURVES          CE
```

Select **TESTS**, **PLATES** or **CURVES** to print the appropriate directory.

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Chapter 5 Running Tests and Recalling Plates

5.1 Running a Test 74
Test Number 74
Number of Samples 75
Plate ID 77
Barcode Scan 78
Reading 78
Variables 79
Editing Areas 79
Curve Fit Output 79
Read Again 83
5.2 Recalling Plates 83

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Once the instrument is switched on and the self-test routines are successfully completed, the Main menu is displayed. This shows the choice of programs.

The appearance of this menu will depend upon the application programs installed. If the Endpoint Program is the only software program installed the Main menu will show only the Endpoint and the Setup options.

```
READY                4:45P  30.06.94
ENDPOINT                      SETUP
```

Select **ENDPOINT** to view the Endpoint menu.

```
ENDPOINT    READY      4:45P  30.06.94
 START  RECALL  UTIL  PROGRAM  CE
```

- **START** Lets you run assay test procedures which have been created using the Program menu
- **RECALL** Lets you recall data obtained from a previous assay test procedure for further processing
- **UTIL** Lets you carry out a manual operation, display the software version number, define calculation modes and set menus
- **PROGRAM** Lets you create and edit assay test procedures.

While the Main menu or the Endpoint menu is displayed, the plate carrier can be moved in and out using the up and down cursor keys. The up key moves the plate carrier in; the down key moves it out.

☞ *Note: If short menus have been selected using the Util menu, up to 8 tests may have been given short-cut keys. These will appear at the ENDPOINT READY display, for example:*

```
ENDPOINT READY          4:45P  30.06.94
TEST1  TEST2  TEST3  TEST4  ENDPOINT
```

The left and right (< >) cursor keys may be used to scroll along the list of tests, and to access tests five to eight. Refer to the Menus section in Chapter 6.

5.1 Running a Test

The **START** options let you operate the Reader using previously created and stored assay test procedures. Several stages are involved in running a test:

Option	Description
Test Number	Select the test to be run
Number of Samples	Select the number of wells on the plate to be assigned to each test
Plate ID	Apply an ID to the plate being used; the Reader will store the plate data under this name. (Only if plate ID prompt was included in the test; refer to the **Plate ID Prompt** section in Chapter 4.)
Barcode Scan	The Reader will scan the barcode and then begin reading the plate. (Only if a barcode scan was included in the test; refer to the **Plate ID Prompt** section in Chapter 4.)
Reading	The test is started: the plate is read and processed, and a results matrix is printed (if specified).
Variables	Enter a figure for each variable which was specified in the ratio or IU equations
Editing Areas	Accept or reject wells with very high or low ODs. (Only if Area Statistics was included in the test; refer to the **Area Statistics** section in Chapter 4.)
Curve Fit Output	Determine if a graph should be calculated and printed, and if it should be saved. (Only if a curve fit option was included in the test; refer to the **Curve Fitting Output Option** section in Chapter 4.)

Test Number

Select **START** to run a test. The Reader asks for the number of the test you want to run.

The name of the displayed test will be shown next to the test number.

```
TEST (00)          4:45P 30.06.94
          DIR   ENTER   CE
```

Select **DIR** to print a list of the stored tests.

Use the keypad or scroll to type in a test number and then select **ENTER**.

When a valid test number has been entered, you must confirm it. For example:

```
TEST (10) RUBELLA      4:45P 30.06.94
                  ENTER   CE
```

Select **ENTER** to confirm and open the test.

Number of Samples

The No. of Samples option lets you assign wells (samples) on the same plate for up to 12 tests. The Reader asks if you want to assign all 96 wells (samples) to the currently open test.

```
NO. OF SAMPLES = 96    4:45P 30.06.94
                  ENTER   CE
```

Select **ENTER** to assign all 96 samples to the test.

To assign less than 96 wells, select **CE**, enter the number of wells and select **ENTER**. Be sure to include the blanks, standards and controls. The section on Running More than One Test on a Plate, below, describes how the Reader assigns the wells.

The Reader asks if you want to assign any more wells to other tests.

```
ANY MORE SAMPLES ?     4:45P 30.06.94
 YES    NO                    CE
```

Select **NO** to leave the rest of the wells unassigned, or select **YES** to assign wells to another test. The section on Leaving Wells Unassigned, below, explains how unassigned wells are discarded.

The Reader asks which test you require. Open the test as described above in the Test Number section.

The Reader asks if you want to assign all the remaining wells to the new test. For example, if you assigned 20 wells to the first test, 76 wells will remain.

```
NO. OF SAMPLES = 76    4:45P 30.06.94
                  ENTER   CE
```

Select **ENTER** to assign all the remaining wells to the new test, or **CE** to assign some to a further test. The Reader will repeat the process until all 96 wells are assigned, or wells are assigned to 12 tests, or you select **NO** at the **ANY MORE SAMPLES** prompt.

If you have left any wells unassigned, or chosen more than one test, the Reader will ask if you want to arrange the test templates in columns or rows.

WELLS IN ?	4:45P 30.06.94
COLUMNS ROWS	CE

See the sections below on how the Reader runs more than one test on a plate, and reduces templates.

Note: If you have arranged your template in rows, you should reduce it in rows. If you have arranged it in columns, you should reduce it in columns. You should not include tests on the same plate if they were arranged in different orientations. For example, one test was arranged in rows and one was arranged in columns.

Note: If you want to see how the template has been reduced, your test must include a Data matrix with labels.

Running More than One Test on a Plate

If you assign wells to more than one test the Reader will assign the wells to the tests in the following way:

If you selected **ROWS**, the first block of wells assigned (to the first test) will be placed in the first wells on the plate, reading along the plate from A1 toA12, B1 to B12 etc.

If you selected **COLUMNS**, the first block of wells assigned will be placed in the first wells on the plate, reading down the plate from A1 to H1, A2 to H2 etc.

The Reader counts all wells along the columns or down the rows, until it reaches the number you specified. It then moves on to the second block of wells for the second test, and so on.

Example

Using the No. of Samples option, 20 wells are assigned to Test1 and 16 wells are assigned to Test2. The templates are arranged in rows.
The Reader will use the first 20 wells, that is, A1 to B8 for Test1. It will use the next 16 wells, that is, B9 to C12 for Test2.

Leaving wells unassigned

If you assign less than 96 wells, the Reader will try to reduce the template, taking into account the wells you defined when you created the test. It does so by removing Test and if necessary Blank wells from the end of the template until the required number of wells is left. Control, Standard and User wells are left in the same positions you defined, relative to the last full column or row on the plate. If it is not possible, using this method, to reduce your template to the correct number, the Reader will print an error message, and it will not reduce the template.

Rows and Columns

If you arrange the template in rows, the Reader will reduce the template upwards from Row H. If you arrange the template in Columns, the Reader will reduce the template backwards from Column 12.

Example

Wells A1 and H11 are Controls and the remaining wells are Test wells. Using the No. of Samples option 48 wells are assigned to Test1, and using the Any More Samples? prompt the rest of the wells are left unassigned. The template is reduced in terms of columns.

The Reader will relocate the second Control well (formerly in H11) to H5, so that it remains in the same position, relative to the end of the plate. Columns 1-6 are read as normal, but columns 7-12 are recorded as unread wells.

Template 1 - Note control wells in positions A1 and H11

	1	2	3	4	5	6	7	8	9	10	11	12
A	C1	T8	T16	T24	T32	T40	T48	T56	T64	T72	T80	T87
B	T1	T9	T17	T25	T33	T41	T49	T57	T65	T73	T81	T88
C	T2	T10	T18	T26	T34	T42	T50	T58	T66	T74	T82	T89
D	T3	T11	T19	T27	T35	T43	T51	T59	T67	T75	T83	T90
E	T4	T12	T20	T28	T36	T44	T52	T60	T68	T76	T84	T91
F	T5	T13	T21	T29	T37	T45	T53	T61	T69	T77	T85	T92
G	T6	T14	T22	T30	T38	T46	T54	T62	T70	T78	T86	T93
H	T7	T15	T23	T31	T39	T47	T55	T63	T71	T79	C2	T94

Template 2 - Note control wells in positions A1 and H5

	1	2	3	4	5	6
A	C1	T8	T16	T24	T32	T39
B	T1	T9	T17	T25	T33	T40
C	T2	T10	T18	T26	T34	T41
D	T3	T11	T19	T27	T35	T42
E	T4	T12	T20	T28	T36	T43
F	T5	T13	T21	T29	T37	T44
G	T6	T14	T22	T30	T38	T45
H	T7	T15	T23	T31	C2	T46

Plate ID

If the plate ID prompt option was included in the test, the Reader prompts you to enter an ID for the plate. This ID will be used to store the plate data so that it can be recalled later, if necessary.

```
PLATE =              4:45P 30.06.94
     YZ ABCD  DIR    ENTER   CE
```

Plate IDs may be up to 12 characters in length and consist of the following characters:

- Numbers 0–9 and a decimal point.
  Enter these using the alphanumeric keypad.
- Upper-case letters, lower-case letters and symbols.
  Enter these in the following way:

  a) Choose upper-, or lower-case letters or the symbols using the up and down direction keys (∧ ∨)

  a) Scroll through the characters on the display with the left and right cursor keys (< >). Enter the character shown by the flashing cursor by pressing the (∧) selection key.

If a plate is already stored with the same ID, the Reader will ask if you want to choose another plate ID, or overwrite this plate ID (and the corresponding plate data). For example:

```
OVERWRITE A3          4:45P 30.06.94
 YES   NO               CE
```

Select **YES** to overwrite the plate data stored under the plate ID, or select **NO** to keep the old plate data and enter a different ID for the new plate.

Barcode Scan

If the Barcode option was included in the assay, the plate carrier will move to the In position and the Reader will scan the barcode. If it reads the barcode successfully, it will go on to start the test.

If it cannot read the barcode it will display an error message, which lets you abort or retry the scan.

If you want to retry the scan, check to make sure that the barcode is correctly positioned and that it is the same type as you defined in the Barcode setup options. Refer to the *Barcode User Manual*.

If you choose to abort, the Reader will ask you to input the plate ID manually. Enter the barcode number in the same way as for a normal plate ID. This is described above.

Reading

Once you have started the plate and input the information required, the test will begin. The Reader will display a **READING...** message, followed by **PROCESSING...** and **SAVING PLATE...** messages.

☞ *Note: While the plate is being read do not block the front of the machine as the plate carrier will move to the Out position so that you can remove the plate.*

Variables

If any variables were specified in any equations, the Reader asks you to enter a figure for each variable. For example:

```
VARIABLE 1 =          4:45P 30.06.94
      e     +/-    ENTER   CE
```

Enter a value for each variable and select **ENTER**.

The Reader will not store the values you enter here. If you want to run the test again using the same variable values, you should make a written record of the values and store them safely.

Editing Areas

If the Area Statistics option was included in the test (refer to the **Area Statistics** section in Chapter 4), the Reader will ask if you want to edit these areas. You may eliminate wells with very high or very low ODs.

```
EDIT AREAS ?          4:45P 30.06.94
 YES    NO
```

Select **NO** if the readings are as expected (these are printed out on the results matrix).

Select **YES** to remove specific well ODs from the calculations.

```
EDIT WELL =           4:45P 30.06.94
                   ENTER   CE
```

Use the alphanumeric keypad to enter a well location and select **ENTER**. The well will be omitted from the calculations. Repeat the process for each well location.

Select **ENTER** on its own to finish editing the areas.

Curve Fit Output

If a curve fit option was included in the test (refer to the Curve Fitting Output Option section in Chapter 4), the Reader will print a table of Standard ODs against concentrations. You may choose to print out a graph of this table.

Several stages are involved in determining the graphical output of a test:

Option	Description
Graph	Determine if a graph should be calculated and printed
Edit Standards	Delete and/or insert the Standards which are used to plot the graph
Save Curve	Store the curve so that it can be recalled for use with another test.

Graph

If the template contained Standards, the Reader asks if you want to print the concentration graph, obtained by plotting the known concentrations of the Standards against their ODs.

```
GRAPH ?              4:45P  30.06.94
 YES    NO    SCALE
```

Select **YES** to calculate and plot a graph and then edit the Standards. Select **NO** to edit the Standards first. Select **SCALE** to set the extent of the graph.

Editing Standards

If the template contained Standards, the Reader will ask if you want to edit them.

```
EDIT STDS ?          4:45P  30.06.94
 YES    NO
```

Select **YES** to edit the Standards. For example:

```
STD. NO. (1 - 3) = ?  4:45P  30.06.94
                    ENTER   CE
```

Use the keypad to enter the number of the Standard you want to edit, and select **ENTER**.

You must then select which wells you want to include in or delete from the curve fit calculations. The Reader will display a prompt for each replicate well of the chosen Standard. For example:

```
O.D. #1  DELETE ?     4:45P  30.06.94
 YES    NO
```

Note: If the replicate has been previously removed from the Standard, the Reader will ask if you want to re-include it.

```
O.D. #1  INCLUDE ?    4:45P  30.06.94
 YES    NO
```

Select **YES** to include or delete the displayed replicate.

When all replicate wells of a particular Standard have been displayed, the **EDIT STDS** prompt will be re-displayed. Edit any remaining Standards as required. Select **NO** at the **EDIT STDS** prompt to finish editing. The display will return to the **GRAPH** prompt and you may choose to print the concentration graph again.

When all the necessary Standards have been edited the Reader re-processes the results.

Too Few Standards

If the curve cannot be calculated because there are not enough Standards, the following error message is displayed:

```
TOO FEW STANDARDS      4:45P  30.06.94
```

The following table summarizes the number of Standards required for the various curves:

Curve Type	Number of Standards
Akima	3
Cubic	4
Linear	1
Michaelis-Menten	3
Polygon	3
Quadratic	3
Quartic	5
Sigmoid (4PLC)	4
Spline	3

Too Many Standards

If a curve contains some Standards which are making it very uneven, the Reader will display a Multi-Valued Curve error message. This gives you the option to edit the Standards and remove the ones which are making the curve uneven.

```
CURVE MULTI-VALUED     4:45P  30.06.94
 PROCEED  ABORT
```

- **ABORT** Abandons the curve fitting processes and continues with the next part of the test
- **PROCEED** Lets you edit the Standards, refer to the Editing Standards section earlier in this chapter.

Sigmoid (4PLC) Process

When a sigmoid (4PLC) curve fit is being processed, the Reader continuously updates the residual error value for the curve. This value measures how well the sigmoid curve is progressing. The lower the number, the better the progress of the sigmoid.

```
RESIDUAL = 0.1093      4:45P  30.06.94
                         ABORT
```

If the residual value is too high you may abort processing and edit the Standards.

Curve Saving

The curve you use can be saved for later use. Refer to the Stored Curve section in Chapter 4.

```
SAVE CURVE ?           4:45P  30.06.94
 YES    NO
```

Select **YES** to save the curve. The Reader asks for a number under which to store the curve.

```
CURVE (00)             4:45P  30.06.94
              DIR    ENTER    CE
```

Up to 20 curves may be stored. To print the directory of stored curves, select **DIR**. Use the keypad to enter a number for the curve and select **ENTER**. If you enter an existing curve number, the Reader asks if you want to overwrite the curve or choose another number. For example:

```
OVERWRITE  CURVE14     4:45P  30.06.94
 YES    NO
```

Once you have entered an available number, you must enter a name.

```
NAME =                 4:45P  30.06.94
     YZ ABCD            ENTER    CE
```

Curve names may be up to 12 characters in length and consist of the following characters:

- Numbers 0–9 and a decimal point.
  Enter these using the alphanumeric keypad.
- Upper-case letters, lower-case letters and symbols.

Enter these in the following way:

a) Choose upper-, or lower-case letters or the symbols using the up and down direction keys (∧ ∨)

a) Scroll through the characters on the display with the left and right direction keys (< >). Enter the character shown by the flashing cursor by pressing the (∧) selection key.

Read Again

At the end of test processing, the option to repeat the test is given.

```
READ AGAIN ?          4:45P  30.06.94
 YES    NO
```

Select **YES** to read the plate again, or **NO** to return to the Endpoint menu.

5.2 Recalling Plates

The Recall option lets you recall data from previously read plates for further processing. Plate data is stored after it has been read (refer to the **Plate ID Prompt** section in Chapter 4). Data from up to 100 plates can be stored in the Reader's memory. The Reader asks for the plate number and the plate ID. For example:

```
PLATE (00) P11          4:45P  30.06.94
              DIR   ENTER   CE
```

Scroll through the plates using the up and down cursor keys. When the correct plate is displayed select **ENTER** to recall the plate data. Select **DIR** to print out a list of plates stored in memory.

When you recall a plate, the Reader asks if you want to process the data using the test with which it was originally run. The number and name of the test under which the plate was read are displayed.

Select **ENTER** to process the plate data with the indicated test.

If you want to process the plate data using a different test, use the keypad to type in a different test number. This option is useful if you want to assess the OD data of the test using a technique not inserted into the original test.

If you enter a number for which no corresponding test exists, the test number prompt will reappear. Re-enter a valid test number.

Recalling plates with default IDs

If you did not assign an ID to a plate but you want to recall the plate data, the Reader will have assigned a default plate ID to the data. To find this ID, print the directory of plate IDs. This will list the date and time that the test was run. You may then be able to work out which plate ID you require.

☞ *Note: As the Reader has a total capacity of 100 plates, default IDs are numbered 0000-0099, unless you have assigned IDs of your own. In this case there will be less default IDs available. Once the Reader reaches its 100 plates capacity it reuses all the available default IDs, overwriting the plate data they hold. It begins at 0000 and works upwards until all the default IDs are used up again. It does not inform you when it begins overwriting old plate data.*

☞ *Note: Partial Plates will have the* ***entire*** *plate recalled and recalculated for only the selected assay.*

WARNING **HAZARD EXISTS PLATE WILL BE RECALLED WITH CURRENTLY SELECTED ASSAY AND NOT ORIGINAL ASSAY.**

Chapter 6 Utilities

The Utility Menu 87
Manual 88
Menus 89
Version 90
Backup 90
C.V. 91
Stats 91
Batch 92
Spectrum 92

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Once the instrument is switched on and the self-test routines are successfully completed, the Main menu is displayed. This shows the choice of programs.

The appearance of this menu will depend upon the application programs installed. If the Endpoint Program is the only software program installed the Main menu will show only the Endpoint and the Setup options.

```
READY                4:45P 30.06.94
ENDPOINT                    SETUP
```

Select **ENDPOINT** to view the Endpoint menu.

```
ENDPOINT    READY     4:45P 30.06.94
 START RECALL  UTIL  PROGRAM  CE
```

- **START** Lets you run assay test procedures which have been created using the Program menu
- **RECALL** Lets you recall data obtained from a previous assay test procedure for further processing
- **UTIL** Lets you carry out a manual operation, display the software version number, define calculation modes and set menus
- **PROGRAM** Lets you create and edit assay test procedures.

While the Main menu or the Endpoint menu is displayed the plate carrier can be moved in and out using the up and down cursor keys. The up key moves the plate carrier in; the down key moves it out.

☞ *Note: If short menus have been selected using the Util menu, up to 8 tests may have been given short-cut keys. These will appear at the ENDPOINT READY display, for example:*

```
ENDPOINT READY          4:45P 30.06.94
TEST1  TEST2 TEST3  TEST4  ENDPOINT
```

The left and right (< >) cursor keys may be used to scroll along the list of tests, and to access tests five to eight. Refer to the Menus section in Chapter 6.

The Utility Menu

The Utility menu lets you carry out a plate read, view the software version number of the Endpoint Program, select long or short menus, and carry out further data calculation.

Select **UTIL** from the Endpoint menu.

```
ENDPOINT READY        4:45P 30.06.94
 OTHER MANUAL  MENUS VERSION   CE
```

Select **OTHER** to access a second Utility menu

```
ENDPOINT READY        4:45P 30.06.94
BACKUP  STATS  BATCH SPECTRUM  CE
```

Note: If you are using a PR 1100 Reader, the Backup option will be replaced by a C.V. option. In the PR 2100 Reader this option is incorporated into the Stats option.

- **MANUAL** Lets you take manual control of the Reader
- **MENUS** Gives you the choice of long or short menus
- **VERSION** Displays the software version number of the currently installed Endpoint Program
- **C.V.** Produces a statistical analysis of plate data
- **BACKUP** Lets you copy data files from hard disk to floppy disk, or from floppy disk to hard disk, or to remove the application from the hard disk
- **STATS** Produces a statistical analysis of plate data
- **BATCH** Recalls a series of stored plates for processing
- **SPECTRUM** Produces a spectral response curve for a well.

Manual

Manual mode lets you operate the Reader manually. Its purpose is to let you to make a manual check of specific results after many plates have been tested.

The Reader asks you to enter the wavelength mode.

```
WAVELENGTH MODE       4:45P 30.06.94
SINGLE  DUAL            CE
```

The Reader may be operated in single or dual wavelength mode. Single wavelength mode involves the use of a test filter only. Dual wavelength mode involves the use of a test filter and reference filter. Refer to the Wavelength Modes section in Chapter 2.

Select **SINGLE** or **DUAL** to specify the wavelength mode.

The Reader asks for the wavelength of the test filter

```
TEST FILTER 405 nm      4:45P 30.06.94
 NEXT   LAST          ENTER   CE
```

Select **ENTER** to confirm the wavelength displayed. Select **LAST** or **NEXT** to scroll backwards or forwards through the available filter wavelengths.

If **DUAL** wavelength mode was selected, the Reader asks for the wavelength of the reference filter. Select the reference filter in the same way as you selected the test filter wavelength.

Reading a Plate

When the filters have been chosen the plate is read. The Reader then displays the results starting at A1. The left and right arrow keys can be used to scroll through the readings for each well.

```
A1 = 0.000          4:45P 30.06.94
 BLANK  CLEAR  PRINT  MATRIX    CE
```

- **BLANK** Zeros the displayed reading and subtracts this value from measurements of subsequent wells
- **CLEAR** Changes a blanked reading back to the original value. All subsequently viewed wells will use air blanking
- **PRINT** Outputs the reading displayed for each well location to a connected printer
- **MATRIX** Prints out an OD matrix.
- **CE** Returns the display to the Main menu.

Menus

The Menus option lets you choose between long or short menus. Long Menus are the normal Endpoint menus. Short Menus involve the use of eight short-cut keys, which can be used to access tests for processing in Start mode. Press **CE** to change the current menu.

```
MENUS ?          4:45P 30.06.94
 SHORT  LONG              CE
```

Select **LONG** or **SHORT** to specify the menus option.

Short Menus

If you select short menus, the Reader will ask which functions keys are to be assigned to which tests. For example:

```
F1= (01) A11          4:45P 30.06.94
              DIR   ENTER   CE
```

Use the keypad to enter the test number to be assigned to the first function key. The test name will be displayed. Select **ENTER** to confirm the displayed test name or **CE** to return to the Menus display. Type in the desired number and press **ENTER**.

Each time the Reader is switched on, the Endpoint menu allow you to choose from the tests you have assigned to short-cut keys. Test 1 to 4 are shown on the display.

```
ENDPOINT READY          4:45P 30.06.94
TEST1  TEST2  TEST3  TEST4  ENDPOINT
```

Tests 5 to 8 can be accessed using the left and right cursor keys.

☞ *Note: All eight function keys must be assigned a test. If, for example, you only have one test to assign, you must assign this test to all eight function keys.*

☞ *Note: Test names may be up to 12 characters long, but only the first seven letters will be used for the short menus display. Make sure that you do not assign two tests with the same first seven letters to short-cut keys.*

Version

The Version option lets you view the software version number of the currently installed Endpoint Program. For example:

```
ENDPOINT VERSION 1.0   4:45P 30.06.94
```

Backup

This option is not available for PR 1100 Readers; refer to The Utility Menu section earlier in this chapter.

The Backup option lets you copy data files from hard disk to floppy disk, or from floppy disk to hard disk, or to remove the application from the hard disk.

```
ENDPOINT READY          4:45P 30.06.94
BACKUP RESTORE UNINSTALL          CE
```

- **BACKUP** Copies the data files held on the Reader hard disk onto floppy disk.

- **RESTORE** Copies the data files held on floppy disk onto the Reader hard disk.
- **UNINSTALL** Removes the Endpoint Program from the Reader hard disk.

☞ *Note: Before using the Backup or Restore commands, make sure that you have a blank disk labeled "Back-up" in the floppy disk drive.*

☞ *Note: The Uninstall command should be used with caution. You will not be able to restore the Endpoint Program onto the hard disk unless you have a copy of the Endpoint disk. If you are upgrading the Endpoint Program, use the Memory upgrade command in the Main menu (refer to the Upgrading from a Previous Version section in Chapter 3).*

C.V.

This option is replaced by the Backup option on PR 2100 Readers, refer to The Utility Menu section earlier in this chapter. To use the C.V. option, refer to the Coefficient of Variation section below.

Stats

The Stats option checks the repeatability of laboratory procedures and calculates statistical data for all stored plates

This option incorporates both Stats and C.V. option on PR 2100 Readers (refer to The Utility Menu section earlier in this chapter).

```
ENDPOINT READY          4:45P  30.06.94
 C.V.   STATS                   CE
```

Coefficient of Variation (C.V.)

The C.V. option checks that the readings from a selected group of plates have an acceptable variation.

You may step through all the plate data files stored in memory and the Reader asks if you want to include each in the CV check. For example:

```
(01) A11          :NO   4:45P  30.06.94
 YES    NO       DIR   FINISH     CE
```

- **NO** Excludes the displayed plate
- **YES** Includes the displayed plate in the calculation
- **DIR** Prints a directory of plates
- **FINISH** Prints the results of the calculation.

Results are printed showing the mean, standard deviation and coefficient of variation for all the valid wells, on all of the plates listed. Blank wells and wells outside the Reader's Over and Under range are excluded.

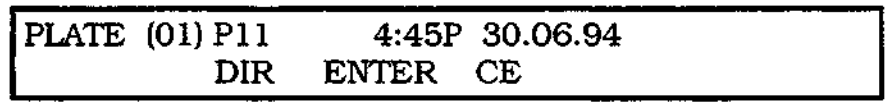
To scroll the Plates directory backwards and forward use the up and down cursor keys.

Stats

The Stats option calculates statistical data for all the plates stored in memory. This option can be used in a similar way to C.V., above, but it operates on areas of the selected plates identified by the Area Statistics option. Refer to the **Area Statistics** section in Chapter 4.

Batch

The Batch option recalls stored plate data and reprocesses it using a different test. **BATCH** operates like **RECALL** (refer to the Recalling Plates section in Chapter 5) but operates on a series of plates rather than a single plate. First you must select a plate.

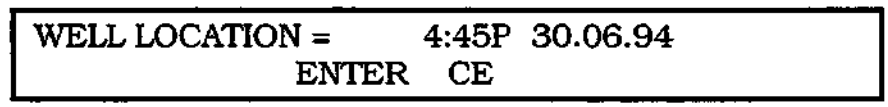


Use the up and down cursor keys, or the alphanumeric keypad to find the plate you need and select **ENTER** to accept it. You must then choose the test to which the plate data will be applied.

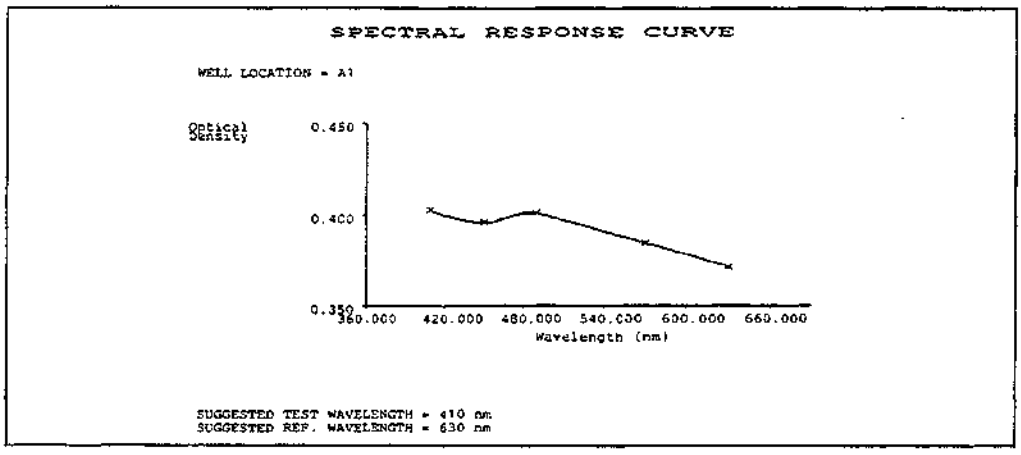
Select **DIR** to print a list of the stored tests. Use the keypad to enter the test number and select **ENTER**. When you have selected all the plate data and tests that you need, select **CE**. The Reader will process all the results and print them on an attached printer.

Spectrum

The Spectrum option lets you produce a spectral response curve for a specified well.



Use the keypad to enter the well location and then select **ENTER**. The Reader will perform readings of the specified well using all the installed filters. The spectral response curve and suggested test and reference filters will then be printed out.



Note: To use the Spectrum option three or more filters must be installed.

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Chapter 7 Computer Control

7.1 Hardware Specification 97
Linking with an IBM-Compatible PC 98
Linking with an Apple Macintosh 99
7.2 Software Specification 100
7.3 Handshaking 100
7.4 Checksum 102
7.5 Computer Control Commands 102
0 Step X/Y 103
1 Start/Stop 104
2 Read Mode 106
3 Filter Select 106
4 Blank Modification 107
5 Print Control 108
6 Display Control 110
7 Computer Control 110
9 Reduced Matrix 110
7.6 Data Format 111
Response with Data 111
Response without Data 113
Uncontrolled Transmission 113

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This chapter contains information on how to interface the Reader to an external PC for data retrieval and control. Example programs are available to assist with computer interfacing (Input/Output Routines).

The information provided in this section is intended for those users who want to write their own applications software. If you are a capable programmer then the versatility of the Reader can be extended even further.

It must be emphasized, however, that this information has been prepared for the computer professional. Therefore, it may be confusing for those not versed in computer communication protocol and peripheral handling.

CAUTION **The applications programmer must take great care to prevent the Reader from starting plate carrier movement when the user is unprepared. This could result in spilled liquids or pinched fingers.**

7.1 Hardware Specification

The Reader computer interface is as specified by EIA RS232C interface standard for a data terminal device. Baud rates between 110 and 9600 may be selected from the Setup menu, refer to the *Microplate Reader User Manual.*

Data characters comprise:

- Start bit
- 8 data bits
- Parity bit
- Stop bit

The following information describes the RS232C cable configuration between the Reader and computers of various different serial outputs. The cable supplied with the Reader conforms to the DB 25 pin serial output specification.

Connections for IBM compatible PCs and Apple Macintosh computers are shown in the sections below.

Linking with an IBM-Compatible PC

The RS232C cable connection between the Reader and an IBM or compatible PC is as follows:

9-pin female – 9-pin female

Reader 9-pin port	Pin Name	PC 9-pin port	Pin Name
2	Receive Data	3	Transmit Data
3	Transmit Data	2	Receive Data
4	Data Terminal Ready	6	Data Set Ready
5	Signal Ground	5	Signal Ground
6	Data Set Ready	4	Data Terminal Ready
7	Request to Send	8	Clear to Send
8	Clear to Send	7	Request to Send

25-pin female – 9-pin female

Reader 25-pin port	Pin Name	PC 9-pin port	Pin Name
3	Receive Data	3	Transmit Data
2	Transmit Data	2	Receive Data
20	Data Terminal Ready	6	Data Set Ready
7	Signal Ground	5	Signal Ground
6	Data Set Ready	4	Data Terminal Ready
4	Request to Send	8	Clear to Send
5	Clear to Send	7	Request to Send

9-pin female – 25-pin female

Reader 9-pin port	Pin Name	PC 25-pin port	Pin Name
2	Receive Data	2	Transmit Data
3	Transmit Data	3	Receive Data
4	Data Terminal Ready	6	Data Set Ready
5	Signal Ground	7	Signal Ground
6	Data Set Ready	20	Data Terminal Ready
7	Request to Send	5	Clear to Send
8	Clear to Send	4	Request to Send

25-pin female – 25-pin female

Reader 25-pin port	Pin Name	PC 25-pin port	Pin Name
2	Receive Data	2	Transmit Data
3	Transmit Data	3	Receive Data
20	Data Terminal Ready	6	Data Set Ready
7	Signal Ground	7	Signal Ground
6	Data Set Ready	20	Data Terminal Ready
4	Request to Send	5	Clear to Send
5	Clear to Send	4	Request to Send

Linking with an Apple Macintosh

The cable supplied with the Reader is for an IBM compatible PC. To link with an Apple Macintosh computer, the cable will have to be modified. The RS232C cable connection between the Reader and a Macintosh is as follows:

9-pin female – Macintosh 8-pin DIN

Reader 9-pin port	Pin Name	8-pin DIN port	Pin Name
2	Receive Data	3	Transmit Data
3	Transmit Data	5	Receive Data
4	Data Terminal Ready	2	Data Set Ready
5	Signal Ground	4	Signal Ground
6	Data Set Ready	1	Data Terminal Ready
7	Request to Send		
8	Clear to Send		

9-pin female – Macintosh 9-pin D type

Reader 9-pin port	Pin Name	9-pin D type port	Pin Name
2	Receive Data	5	Transmit Data
3	Transmit Data	9	Receive Data
4	Data Terminal Ready	7	Data Set Ready
5	Signal Ground	3	Signal Ground
6	Data Set Ready		
7	Request to Send		
8	Clear to Send		

7.2 Software Specification

Software is defined by the standard ASCII character set together with the following specific control characters for handshaking:

STX (02H)	Start of text	Begins transmission
ETX (03H)	End of text	Terminates transmission
ENQ (05H)	Enquiry	Sent by Reader to indicate ready to transmit
ACK (06H)	Acknowledge	Used to indicate proper reception of transmission
XON (11H)	Start	Start transmitting data
XOFF(13H)	Stop	Stop transmitting data
NAK (15H)	Not Acknowledge	Used to indicate faulty transmission

7.3 Handshaking

The RS232C port is bi-directional when used in computer control mode. The DSR/DTR handshake signals as well as software handshake are used to maintain communication status and synchronisation. Computer control is established as follows:

	COMPUTER	Reader
1	DSR asserted	
2	STX COMMAND ARGUMENTS	
	checksum ETX	
	Sends command character string	
3		ACK/NAK
		Indicates proper/improper reception
4		Executes task
5		ENQ
		Asks if computer is ready to receive answer

6	ACK/NAK Indicates ready/not ready to receive	
7		STX RESULTS/ANSWER checksum ETX Sends answer
8	ACK/NAK Indicates proper/improper reception	

Steps 2 to 8 are repeated for subsequent transmissions provided the data link remains connected. Results may require more than a single transmission in which case steps 5 to 8 must be repeated. If in either step 6 or step 8 the computer has not acknowledged receipt of data (NAK), the Reader will return to step 5 and retransmit the results.

The following example illustrates the data stream for initiating computer control mode for the Reader. Hardware handshaking (step 1) has been omitted.

Computer	STX 7 ETX		AC K	ACK
HEX	02 37 03		06	06
Reader		ACK ENQ STX	ACK ETX	
HEX		06 05 02	06 03	

Note: The example shown above does not use checksums. Checksums are optional and are initiated by the "72" command.

7.4 Checksum

The Reader is equipped with a Fletcher's checksum algorithm to protect against any communication problems.

The checksum is calculated as follows:

sum1 = 0
sum2 = 0
for i from 1 to message length do
    sum1 = (sum1 + message [i]) modulo 255
    sum2 = (sum2 + sum 1) modulo 255
end for
checksum = sum2*255 + sum1

This checksum is transmitted as a 4 digit hexadecimal (base16) number in ASCII format.

Example: Normal command STX 5 0 ETX

character	ASCII code	sum1	sum2
		0	0
STX	2	2	2
"5"	53	55	57
"0"	48	103	160

Checksum = 160 x 255 + 103 = 40903 = $9FC7_{16}$

The command therefore becomes: STX 5 0 9 F C 7 ETX

Note: The Reader data format includes a checksum whether this command is sent or not.

7.5 Computer Control Commands

The COMMAND data string has the following format:

STX [A] {ARGUMENTS} {Checksum} ETX

where:

- STX is the ASCII Start of Text character
- A is the ASCII character 0,1,2,3,4,5,6,7,9
- ARGUMENTS are ASCII alphanumerics needed by the particular command
- ETX is the ASCII End of Text character
- Checksum (optional, see above).

Note: Make sure that the computer only sends the above codes. Some computers automatically send CR and LF, these must be suppressed.

There are 10 basic commands which are described by the ASCII numbers 0 to 9. These have been supplemented by additional commands which are denoted by two ASCII numbers as subsets of the core command. The basic commands are:

0 = Step (X/Y)
1 = Start/Stop
2 = Read Mode
3 = Filter Select
4 = Blank Modification
5 = Printer Control
6 = Display Control
7 = Computer Control
9 = Reduced Matrix

0 Step X/Y

STX 0 [A] {B C} ETX

A is an ASCII number 0, 1, or 2:

0 = Step only in the X direction
1 = Step only in the Y direction
2 = Step both X and Y

B is the number of steps in the first direction

C is the number of steps in the second direction, that is, the Y direction when A = 2.

This command is analogous to an MR700 instruction for incrementing plate movement but in this case will step through well locations stored in memory.

The arguments B and C are derived by finding the ASCII equivalent of adding 30H (48 decimal) to the number of steps required. Consequently if an ASCII character, A, was the argument, 17 steps would result. If the number of steps is more than is left in the row or column then the pointer will return to the equivalent of the home position in memory and continue to step until complete.

If B or C is missing, then the default value of 1 results.

1 Start/Stop

STX 1 [A] ETX

A is an ASCII number 0 or 1:

0 = STOP
1 = START

STOP: stops operation and return plate carrier to the Out position.

START will start reading operation in the mode currently selected.

Shake Commands

STX 1 2 n n ETX

Shake the plate for 1 to 59 seconds and then read the plate in the mode currently selected.

n n is the shake period in seconds (entered as a decimal value)

For example, STX 1 2 3 4 ETX — shake for 34 seconds
STX 1 2 0 8 ETX — shake for 8 seconds

STX 1 3 n n ETX

Shake the plate for 1 to 59 seconds without reading.

n n is the shake period in seconds entered as a decimal value

Transmit Stored Data by Plate Number

STX 1 4 n n ETX

This command recalls data from memory by plate number.

n n is the plate storage number (from plate directory), entered as a decimal value between 0 and 99.

Following this command, OD data will be transmitted in the same data format as if the plate had just been read.

Transmit Stored Data by Plate ID

STX 1 7 n n n n n n n n n n n n ETX

This command recalls data from memory by plate ID.

n n n n n n n n n n n n is the plate ID number (from plate directory), entered as letters, numbers or special characters

Following this command, OD data will be transmitted in the same data format as if the plate had just been read.

Switch the Lamp On/Off

STX 1 5 ETX Switch the lamp on.

STX 1 6 ETX Switch the lamp off.

Users should exercise caution when using these commands; allowing the lamp to remain on unnecessarily will shorten its life.

Shake Mode Command

This command sets the Shake mode.

STX 1 8 **n** ETX

n is the ASCII number 1 to 4 denoting shake modes with different frequencies and amplitudes.

1 = Frequency = 14Hz (+/– 2Hz). Amplitude = 1.7mm
2 = Frequency = 9Hz (+/– 2Hz). Amplitude = 3.0mm
3 = Frequency = 8Hz (+/– 2Hz). Amplitude = 3.0mm
4 = Sets Custom Shake mode which is defined using commands 1 9 and 1 A.

Custom Shake Mode Frequency Command

This command sets the frequency of the Custom Shake operation.

STX 1 9 **nn** ETX

nn is a decimal number denoting the frequency.
Minimum: 5 Hz. Maximum: 20 Hz.

Example To set the frequency to 14Hz: STX 1 9 **14** ETX

Custom Shake Mode Amplitude Command

This command sets the amplitude of the Custom Shake operation.

STX 1 A **nnnn** ETX

nnnn is a decimal number denoting the amplitude.
Minimum: 1.0 mm. Maximum: 10.0mm.

Example To set the amplitude to 2.0mm

STX 1 A **2.0** ETX

Plate Retraction Command

This command returns the plate to the In position.

STX 1 B ETX

2 Read Mode

STX 2 [A] ETX

A is an ASCII number 0, 1, 2 or 3

0 Normal read mode: One OD value is transmitted for each well

1 Five filter read mode: ODs for each of the five filters are transmitted for each well

2 Transmit result for single well (providing normal read mode has been set, A =0): In normal operation the whole plate is read and all results are transmitted to the computer. Setting A = 2 will allow data from a single well to be transmitted, providing the five filter mode has not been previously set

3 Transmit data as percent transmission rather than optical density: Transmission data is sent to five decimal places. The lamp remains on following the read (and must be switched off using the STX 1 6 ETX command). Using this command, data from a complete plate can be transmitted in dual wavelength mode in under 10 seconds. This is useful for Endpoint tests.

4 This command sets the repeatability mode. Using the repeatability mode you can specify that each reading is repeated a number of times and averaged into a single result.

For example, to set the repeatability mode to 1 repeat only:
STX 2 4 1 ETX

5 The Over limit is used to set a range within which ODs should fall. ODs above the limit will not be processed. The limit is also used as an Under limit to set the negative limit of the range.

For example, to set the Over limit to 3.500 and the Under limit to -3.500:STX 2 5 **3.500** ETX

3 Filter Select

STX 3 [A B] ETX

A is an ASCII number 0, 1 or 2

0 = Set sample filter
1 = Set reference filter
2 = Set mode

B is an ASCII number 1 to 6 which identifies each filter. Alternatively, if A has been set to 2 (set mode), the ASCII numbers 0 and 1 represent single and dual wavelength modes respectively.

Filters Installed

STX 3 3 ETX

This command transmits the list of filter wavelengths as installed in the instrument. The system supplies the data in the form:

410 450 490 570 630 ETX (separated by spaces)

4 Blank Modification

STX 4 [A] {B C D} .... ETX

A is an ASCII number 0 to 5

0 Reset blank value to zero (no additional arguments necessary)

1 Reset blank matrix to zero

2 Defined blank matrix will be used, A1 is set as blank initially if matrix is not previously defined

3 The next and subsequent readings are blanks until reset

4 Set matrix of blank values defined by the following arguments (explained below)

5 The previous reading is used as blank value for subsequent readings (this command resets argument A = 3)

B is the column indicator in the matrix command defined by the ASCII numbers 1 to 9, and the following special characters:
: = 10 ; = 11 < = 12

C is an ASCII number or symbol 1 to ? (see table below for indication of blank wells) affecting the wells D through A

D is an ASCII number or symbol 1 to ? (see table below for indication of blank wells) affecting the wells H through E

ASCII	WELL H/D	WELL G/C	WELL F/B	WELL E/A
1	BLANK	-	-	-
2	-	BLANK	-	-
3	BLANK	BLANK	-	-
4	-	-	BLANK	-
5	BLANK	-	BLANK	-
6	-	BLANK	BLANK	-
7	BLANK	BLANK	BLANK	-
8	-	-	-	BLANK
9	BLANK	-	-	BLANK
:	-	BLANK	-	BLANK

;	BLANK	BLANK	-	BLANK
>	-	-	BLANK	BLANK
=	BLANK	-	BLANK	BLANK
<	-	BLANK	BLANK	BLANK
?	BLANK	BLANK	BLANK	BLANK

As an example, STX 44221 ETX would cause blanking in column 2 on wells C and H.

Each {B,C,D} set represents one row. Further {B,C,D} sets can be added in the command to set additional blanks, that is, STX 44221554 ETX will set blanks in column 2 on wells C and H, and also column 5 on wells B, D and F.

Arguments A = 0, 3, and 5 should be used collectively when data from individual wells are transmitted singly as in MANUAL mode (STX 2 A = 2).

Similarly arguments A = 1, 2 and 4 are used in normal read mode (STX 2 A = 0) since they affect a matrix of blanks across the plate.

If argument A = 3 is set in MANUAL mode, blanks will be assigned for each well position addressed until the command is reset by argument A = 5.

As the plate is read, when a new blank well is encountered, the blank value is updated and is subtracted from all subsequent wells.

In normal read mode up to a maximum of 32 blanks can be set across the plate.

5 Print Control

STX 5 [A] {character string} ETX

A is the ASCII number 0 or 1

0 Disable printer. Printer will not print until the computer resets or relinquishes control

1 Enable printer. If a character string follows (maximum 196 characters) it will be printed, otherwise the printer will function as usual

Print control characters can also be sent, refer to the printer manual.

Print Installed Files

STX 5 2 ETX

This command transmits codes indicating installed programs.

Data takes the form:
n n n n n n n n n n n n n n n n n n n ETX (separated by spaces)
where n =

0 means page unoccupied
1 indicates page accessing Reader software
2 indicates inaccessible program cartridge
8 = Barcode
E= Agglutination
H= Kinetics

Test Backup Commands

STX 5 3 ETX

This command will cause the Reader to transmit all tests stored in memory. The data will be variable in length but the end will be marked by an ETX character. Normal Reader communications protocol should be observed.

STX 5 4 ETX

This command will replace all tests stored in the Reader memory with tests downloaded from a computer. Once this command has been acknowledged, data follows, terminated by an ETX signal. Receipt of the data is acknowledged following ENQ sent by the computer. The Reader will respond by transmitting STX ACK ETX if data has been received successfully.

Note: Users are advised to obtain data transfer software from the Reader supplier. The above commands use large data sets and the data format is non-standard. Writing successful programs to handle these data sets is not straightforward.

6 Display Control

STX 6 {control/character string} ETX

This command allows the computer to access the 2 x 40 LCD display. It recognises the following special control characters:

CHARACTER	ASCII	DESCRIPTION
BS	08H	Writing position moves left one character.
HT	09H	Writing position moves right one character.
LF	0AH	Display is cleared, writing position moves to right end of display
CR	0DH	Writing position moves to left end of the display.

7 Computer Control

STX 7 [A] ETX

This command acts as a toggle, beginning or ending computer control. The Reader will not accept other commands from the computer until control is restarted.

A is the ASCII number 0, 1 or 2; where:

0 = Stop
1 = Start
2 = Start with Checksums

STX 7 1 ETX

This command is used to begin computer control once the data link is established.

STX 7 0 ETX

This command is used to end computer control.

STX 7 ETX

This command acts as a toggle.

STX 7 2 ETX

This command begins computer control and enables checksums.

9 Reduced Matrix

STX 9 A B ETX

A is the number of columns in the reduced matrix

B is the number of rows in the reduced matrix

This command can be used to reduce the size of the matrix. For example if only 40 wells are needed for a test, a reduced matrix of 5 columns by 8 rows could be used

7.6 Data Format

The following conventions are used for data format:

b represents a space (ASCII 20H).

xx represents a numeric value, 0 - 12, of the X well position right justified and with leading space if necessary.

y represents the Y position (@ is the home Y position, ASCII 40H)

q represents the sign of the number. Space for positive values and minus sign (-) for negative (ASCII 2DH).

u represents an underline (ASCII 5FH).

cr represents carriage return (ASCII ODH).

r represents the number of the reference filter used (* means reference filter not applicable).

s represents sample filter number.

represents numeric value.

Response with Data

There are three different possibilities for data streams that may be sent by the Reader, when under computer control.

Single Value Read Mode

This occurs when the single filter mode is selected and a single well read and transmit command has been issued. The following data stream will result:

x x b y b b b q # . # # # cr ETX

Normal Read Mode

This is normal operation where the whole plate is read after the start command has been issued. The data stream is divided into two parts: a header and a body. The header is transmitted first:

MR700 data format:

1 b M O D E b R E F b S A M c ETX
2 b S N G L b b * b b b s cr ETX
or b D U A L b b r b b b s cr ETX
3 T H R S H L D = b 1 . 0 0 cr ETX
4 C A L = b b b b b 1 . 0 0 cr ETX
5 D A T E : u u u u u u u u u cr ETX
6 O P E R : u u u u u u u u u cr ETX

Note: Lines 3 to 6 do not contain valid data and are only included to maintain compatibility with existing MR700 software.

Extended and Reader data format:

1 b M O D E b R E F b7 S A M cr ETX

2 b S N G L b b * b b b s cr ETX3 T I M E : # # : # # # cr ETX

4 D A T E : # # . # # . # # cr ETX

5 I D : # # # # # # # # # # # # # cr ETX

6 O P E R : u u u u u u u u u u cr ETX

The body consists of twelve data blocks representing the twelve columns of a microplate. The data blocks consist of the following:

1 x x b y b b b q # . # # # cr

2 x x b y b b b q # . # # # cr

:

:

8 x x b y b b b q # . # # # cr

9 ETX

There are three possible exceptions to the #. # # # field. If a well is blank or out of range, the following is transmitted:

x x b y b b b b B L A N K cr

or x x b y b b b b b O V E R cr

or x x b y b b b b U N D E R cr

Five Filter Read Mode

This occurs when the five filter mode has been specified before the start command has been issued. The header is the same as before and the body also consists of twelve data blocks each representing the columns of a microplate.

1	#.###	#.###	#.###	#.###	#.###
	filter 1	filter 2	filter 3	filter 4	filter 5
2	#.###	#.###	#.###	#.###	#.###
3	#.###	#.###	#.###	#.###	#.###
	:				
	:				
8	#.###	#.###	#.###	#.###	#.###
9	ETX				

☞ *Note: If a five filter read value is over range, the value 9.999 is transmitted.*

Response without Data

In all instances, where a command requires no data from the Reader, the microplate will issue one of the following data streams.

STX ACK ETX (indicating it has complied and is ready for further commands)

STX NAK (A) ETX (indicating that it cannot comply, with A as one of the following error codes)

ERROR	DESCRIPTION
1	Argument out of range in step command
2	Argument out of range in start/stop command
3	Argument out of range in read command
4	Argument out of range in filter command
5	Filter command out of range
6	Argument out of range in print command
7	Command out of range
8	No STX at start of command string
A	Blank command out of range
B	Blank argument out of range

Uncontrolled Transmission

The Reader can send a results matrix to the parallel printer port and serial RS232C port simultaneously. Data is sent to the RS232C port provided that the Reader detects the presence of the computer.

If the above condition is met, the RS232C port will transmit data uncontrolled without the normal handshake. The data format is identical to that described earlier. You can readily copy the input from the computer's RS232C port to a data file and examine or edit the file when transmission is complete.

To prevent the computer's input buffer becoming overloaded and data being lost, software handshaking using XON/XOFF should be used. When an XOFF (character 13H) control code is transmitted, the Reader stops transmitting data and waits for the host to send an XON (character 11H) control code.

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Appendix A: Examples of Plate Formats

Single Well Standards in Rows 117
Single Well Standards in Columns 117
Double Well Standards in Rows, Replicates in Columns 118
Double Well Standards in Columns, Replicates in Rows 118
Triple Well Standards in Rows, Replicates in Columns 119
Triple Well Standards Columns, Replicates in Rows 119
Alternative Format: Single Well Standards in Rows 120
Alternative Format: Quadruple Well Standards in Columns, Replicates in Rows 120

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Single Well Standards in Rows

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
B	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
C	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24
D	T25	T26	T27	T28	T29	T30	T31	T32	T33	T34	T35	T36
E	T37	T38	T39	T40	T41	T42	T43	T44	T45	T46	T47	T48
F	T49	T50	T51	T52	T53	T54	T55	T56	T57	T58	T59	T60
G	T61	T62	T63	T64	T65	T66	T67	T68	T69	T70	T71	T72
H	T73	T74	T75	T76	T77	T78	T79	T80	T81	T82	T83	T84

Single Well Standards in Columns

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	T1	T9	T17	T25	T33	T41	T49	T57	T65	T73	T81
B	S2	T2	T10	T18	T26	T34	T42	T50	T58	T66	T74	T82
C	S3	T3	T11	T19	T27	T35	T43	T51	T59	T67	T75	T83
D	S4	T4	T12	T20	T28	T36	T44	T52	T60	T68	T76	T84
E	S5	T5	T13	T21	T29	T37	T45	T53	T61	T69	T77	T85
F	S6	T6	T14	T22	T30	T38	T46	T54	T62	T70	T78	T86
G	S7	T7	T15	T23	T31	T39	T47	T55	T63	T71	T79	T87
H	S8	T8	T16	T24	T32	T40	T48	T56	T64	T72	T80	T88

Double Well Standards in Rows, Replicates in Columns

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
C	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
D	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
E	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24
F	T13	T14	T15	T16	T17	T18	T19	T20	T21	T22	T23	T24
G	T25	T26	T27	T28	T29	T30	T31	T32	T33	T34	T35	T36
H	T25	T26	T27	T28	T29	T30	T31	T32	T33	T34	T35	T36

Double Well Standards in Columns, Replicates in Rows

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	T1	T1	T9	T9	T17	T17	T25	T25	T33	T33
B	S2	S2	T2	T2	T10	T10	T18	T18	T26	T26	T34	T34
C	S3	S3	T3	T3	T11	T11	T19	T19	T27	T27	T35	T35
D	S4	S4	T4	T4	T12	T12	T20	T20	T28	T28	T36	T36
E	S5	S5	T5	T5	T13	T13	T21	T21	T29	T29	T37	T37
F	S6	S6	T6	T6	T14	T14	T22	T22	T30	T30	T38	T38
G	S7	S7	T7	T7	T15	T15	T23	T23	T31	T31	T39	T39
H	S8	S8	T8	T8	T16	T16	T24	T24	T32	T32	T40	T40

Triple Well Standards in Rows, Replicates in Columns

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
C	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
D	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
E	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
F	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
G	-	-	-	-	-	-	-	-	-	-	-	-
H	-	-	-	-	-	-	-	-	-	-	-	-

Triple Well Standards Columns, Replicates in Rows

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S1	T1	T1	T1	T9	T9	T9	T17	T17	T17
B	S2	S2	S2	T2	T2	T2	T10	T10	T10	T18	T18	T18
C	S3	S3	S3	T3	T3	T3	T11	T11	T11	T19	T19	T19
D	S4	S4	S4	T4	T4	T4	T12	T12	T12	T20	T20	T20
E	S5	S5	S5	T5	T5	T5	T13	T13	T13	T21	T21	T21
F	S6	S6	S6	T6	T6	T6	T14	T14	T14	T22	T22	T22
G	S7	S7	S7	T7	T7	T7	T15	T15	T15	T23	T23	T23
H	S8	S8	S8	T8	T8	T8	T16	T16	T16	T24	T24	T24

Alternative Format: Single Well Standards in Rows

	1	2	3	4	5	6	7	8	9	10	11	12
A	*	*	*	*	*	*	*	*	*	*	*	*
B	*	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	*
C	*	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	*
D	*	T11	T12	T13	T14	T15	T15	T17	T18	T19	T20	*
E	*	T21	T22	T23	T24	T25	T26	T27	T28	T29	T30	*
F	*	T31	T32	T33	T34	T35	T36	T37	T38	T39	T40	*
G	*	T41	T42	T43	T44	T45	T46	T47	T48	T49	T50	*
H	*	*	*	*	*	*	*	*	*	*	*	*

Alternative Format: Quadruple Well Standards in Columns, Replicates in Rows

	1	2	3	4	5	6	7	8	9	10	11	12
A	*	*	*	*	*	*	*	*	*	*	*	*
B	*	S1	S1	S1	S1	T1	T1	T1	T1	-	-	*
C	*	S2	S2	S2	S2	T2	T2	T2	T2	-	-	*
D	*	S3	S3	S3	S3	T3	T3	T3	T3	-	-	*
E	*	S4	S4	S4	S4	T4	T4	T4	T4	-	-	*
F	*	S5	S5	S5	S5	T5	T5	T5	T5	-	-	*
G	*	S6	S6	S6	S6	T6	T6	T6	T6	-	-	*
H	*	*	*	*	*	*	*	*	*	*	*	*

\- denotes unused wells

* denotes unused wells in alternative format

Appendix B: Sample Printouts

Directory of Tests 123
Directory of Plates 124
Directory of Curves 125
Results Matrix: Labelled OD Matrix 125
Threshold Data: Test Printout 126
Threshold Data: Results Printout 127
Curve Fit Data: Test Printout 128
Curve Fit Data: Results Printout 129
Curve Fit Data 130
  Linear Power Fit 130
  Quadratic Power Fit 130
Curve Fit Data 131
  Cubic Power Fit 131
  Quartic Power Fit 131
Curve Fit Data 132
  Cubic Spline Fit 132
  Polygon Fit 132
Curve Fit Data 133
  Sigmoid Fit 133
  Michaelis-Menten Fit 133
Curve Fit Data 134
  Akima Fit 134
Ratio Data: Test Printout 135
Ratio Data: Results Printout 136
Difference Data: Test Printout 137
Difference Data: Results Printout 138
Area Statistics: Test Printout 139
Area Statistics: Results Printout 139

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Directory of Tests

DMPL Lab #JF Printed at 14:45 on 22.06.94 Page 1

TEST DIRECTORY

	TEST NAME:		TEST NAME
00	READ	50.	
01	READ1	51.	
02	HEPATITUS	52.	
03	HIV-1	53.	
04	HIV-2	54.	
05	SINGLE	55.	
06	READ3	56.	
07	THRESH	57.	
08	DIFF	58.	
09	RATIO	59.	
10	CURVE1	60.	
11	CURVE2	61.	
12	CURVE3	62.	
13	CURVE4	63.	
14	CURVE5	64.	
15	CURVE6	65.	
16	CURVE7	66.	
17	CURVE8	67.	
18	CURVE9	68.	
19	CURVE10	69.	
20	DUAL	70.	
21		71.	
22		72.	
23		73.	
24		74.	
25		75.	
26		76.	
27		77.	
28		78.	
29		79.	
30		80.	
31		81.	
32		82.	
33		83.	
34		84.	
35		85.	
36		86.	
37		87.	
38		88.	
39		89.	
40		90.	
41		91.	
42		92.	
43		93.	
44		94.	
45		95.	
46		96.	
47		97.	
48		98.	
49		99.	

Directory of Plates

DMPL Lab #JF Printed at 14:45 on 22.06.94 Page 2

PLATE DIRECTORY

	PLATE ID	TIME	DATE		PLATE ID	TIME	DATE
00	0000	16:14	18.08.94	50	A	15:03	04.08.94
01	0001	22:54	11.09.94	51	A	15:08	04.08.94
02				52	AA	15:12	04.08.94
03				53	AAA	15:15	04.08.94
04				54	A	15:16	04.08.94
05				55	0055	15:19	04.08.94
06				56	0056	15:25	04.08.94
07				57	0057	15:34	04.08.94
08				58	0058	15:37	31.08.94
09				59	0059	15:43	31.08.94
10				60	0060	16:42	31.08.94
11				61	0061	17:12	31.08.94
12				62	0062	17:13	31.08.94
13				63	0063	17:13	01.09.94
14				64	0064	08:43	01.09.94
15				65	0065	08:45	01.09.94
16				66	0066	09:03	01.09.94
17				67	0067	09:04	01.09.94
18				68	0068	09:09	01.09.94
19				69	0069	09:11	01.09.94
20				70	0070	09:52	01.09.94
21				71	0071	09:57	01.09.94
22				72	0072	09:58	01.09.94
23				73	0073	10:00	01.09.94
24				74	0074	10:02	01.09.94
25				75	0075	10:18	01.09.94
26				76	0076	10:18	01.09.94
27				77	0077	10:42	01.09.94
28				78	0078	10:43	01.09.94
29				79	0079	10:45	01.09.94
30				80	0080	10:47	01.09.94
31				81	0081	10:48	01.09.94
32				82	0082	10:50	01.09.94
33				83	0083	10:52	01.09.94
34				84	0084	11:18	01.09.94
35				85	0085	11:19	01.09.94
36				86	0086	11:56	01.09.94
37				87			
38				88			
39				89			
40				90			
41				91			
42				92			
43				93			
44				94			
45	0045	15:18	03.08.94	95			
46	0046	15:19	03.08.94	96			
47	0047	14:48	04.08.94	97			
48	A	14:50	04.08.94	98			
49	A	14:58	04.08.94	99			

Directory of Curves

DMPL Lab #JF Printed at 14:45 on 22.06.94 Page 3

CURVE DIRECTORY

00 A
01 A
02 B
03 C
04 D
05 E
06 F
07 G
08 H
09 I
10 J
11 K
12
13
14
15
16
17
18
19

Results Matrix: Labelled OD Matrix

	1	2	3	4	5	6	7	8	9	10	11	12	
A	B1 BLANK	S4 0.888	PC1 1.707	T1 0.264	T5 0.001	T9 0.001	T13 1.337	T17 1.354	T21 0.063	T25 0.000	T29 0.000	T33 0.845	A
B	B1 BLANK	S4 0.976	PC1 0.277	T1 0.000	T5 0.919	T9 1.483	T13 0.002	T17 0.066	T21 1.239	T25 0.000	T29 0.933	T33 0.796	B
C	S1 -0.005	S5 0.946	PC2 0.067	T2 1.459	T6 0.076	T10 0.283	T14 0.306	T18 1.621	T22 0.066	T26 0.863	T30 1.120	T34 0.064	C
D	S1 -0.004	S5 1.186	PC2 1.199	T2 0.076	T6 0.308	T10 0.051	T14 1.356	T18 0.002	T22 0.059	T26 0.792	T30 0.346	T34 0.001	D
E	S2 0.056	S6 1.626	NC1 1.159	T3 1.098	T7 0.945	T11 1.337	T15 1.389	T19 0.815	T23 0.002	T27 1.314	T31 0.002	T35 1.409	E
F	S2 0.061	S6 1.648	NC1 0.071	T3 1.726	T7 0.720	T11 1.090	T15 1.109	T19 1.381	T23 1.622	T27 0.001	T31 1.301	T35 0.338	F
G	S3 0.306	S7 2.103	NC2 0.886	T4 0.268	T8 1.443	T12 0.241	T16 0.000	T20 0.064	T24 0.002	T28 0.256	T32 0.043	T36 0.312	G
H	S3 0.312	S7 1.830	NC2 0.001	T4 1.277	T8 1.077	T12 1.161	T16 0.050	T20 0.257	T24 0.970	T28 0.854	T32 0.079	T36 0.964	H
	1	2	3	4	5	6	7	8	9	10	11	12	

***** INDICATES VALUE OUT OF RANGE
INDICATES COMBINED DATA

Threshold Data: Test Printout

DMPL Lab #JF Printed at 14:45 on 22.06.94 Page 6

TEST NO: 01 TEST NAME: MRX1

```
PASSWORD              :
WAVELENGTH MODE       : SINGLE
TEST FILTER           : 410nm
REF. FILTER           :  *
PLATE ID PROMPT       : NO
O.D. MATRIX           : YES
SHAKE                 : NO
BLANK MODE            : AVERAGE
```

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC1	T1	T9	T17	T25	T33	T41	T49	T57	T65	T73	T81
B	NC2	T2	T10	T18	T26	T34	T42	T50	T58	T66	T74	T82
C	NC3	T3	T11	T19	T27	T35	T43	T51	T59	T67	T75	T83
D	NC4	T4	T12	T20	T28	T36	T44	T52	T60	T68	T76	T84
E	CO1	T5	T13	T21	T29	T37	T45	T53	T61	T69	T77	T85
F	CO2	T6	T14	T22	T30	T38	T46	T54	T62	T70	T78	T86
G	PC1	T7	T15	T23	T31	T39	T47	T55	T63	T71	T79	T87
H	PC2	T8	T16	T24	T32	T40	T48	T56	T64	T72	T80	T88

```
Q.C. EQUATIONS  :

AREA STATISTICS : NO
```

THRESHOLD.

```
  -   EQN             : CO*0.9
  +   EQN             : CO*1.1
 ++   EQN             : CO*1.5
 +++  EQN             : CO*2
NO.   OF SEGMENTS     : 1
  -   LABEL           : -
  0   LABEL           : 0
  +   LABEL           : +
 ++   LABEL           : ++
 +++  LABEL           : +++
```

Threshold Data: Results Printout

DMPL Lab #JF Printed at 14:45 on 22.06.94 Page 7

TEST NO. : 01 W/L MODE : SINGLE DATE : 18.03.94
TEST NAME : MRX1 TEST FILTER : 410 nm TIME : 16:14
PLATE : PLATE1 REF. FILTER : * OPERATOR :

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.034	0.922	1.741	0.298	0.035	0.035	1.371	1.388	0.097	0.034	0.034	0.879
B	0.034	1.010	0.311	0.034	0.953	1.517	0.036	0.100	1.273	0.034	0.967	0.830
C	0.029	0.980	0.101	1.493	0.113	0.317	0.340	1.655	0.100	0.897	1.154	0.098
D	0.030	1.220	1.233	0.110	0.342	0.085	1.390	0.036	0.093	0.826	0.380	0.035
E	0.090	1.660	1.193	1.132	0.979	1.371	1.423	0.849	0.036	1.348	0.036	1.443
F	0.095	1.682	0.105	1.760	0.754	1.114	1.143	1.415	1.656	0.035	1.335	0.372
G	0.340	2.137	0.920	0.302	1.477	0.275	0.034	0.098	0.036	0.290	0.077	0.346
H	0.346	1.864	0.035	1.311	1.111	1.195	0.084	0.291	1.004	0.888	0.113	0.998

THRESHOLD MATRIX

```
+++ EQN = CO*2
        = 0185
++  EQN = C0*1.5
        = 0.139
 +  EQN = CO*1.1
        = 0.102
 -  EQN = CO*0.9
        = 0.083
```

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC1	+++	+++	+++	-	-	+++	+++	0	-	-	+++
B	NC2	+++	+++	-	+++	+++	-	0	+++	-	+++	+++
C	NC3	+++	0	+++	+	+++	+++	+++	0	+++	+++	0
D	NC4	+++	+++	+	+++	0	+++	-	0	+++	+++	-
E	CO1	+++	+++	+++	+++	+++	+++	+++	-	+++	-	+++
F	CO2	+++	+	+++	+++	+++	+++	+++	+++	-	+++	+++
G	PC1	+++	+++	+++	+++	+++	-	0	-	+++	-	+++
H	PC2	+++	-	+++	+++	+++	0	+++	+++	+++	+	+++

Curve Fit Data: Test Printout

```
   DMPL Lab #JF          Printed at 14:45  on 22.06.94          Page 8
                        TEST NO: 02        TEST NAME: MRX2
PASSWORD              :
WAVELENGTH MODE       : DUAL
TEST FILTER           : 405nm
REF. FILTER           : 630nm
PLATE ID PROMPT       : YES
O.D. MATRIX           : YES
SHAKE                 : NO
BLANK MODE            : AVERAGE

    1     2     3     4     5     6     7     8     9     10    11    12
A   B1    S4    T1
B   B1    S4    T1
C   S1    S5    T2
D   S1    S5    T2
E   S2    S6    T3
F   S2    S6    T3
G   S3    S7    T4
H   S3    S7    T4

Q.C. EQUATIONS        :
AREA STATISTICS       : NO

CURVE FIT.

CONC. OF S1       : 1.000 ug/ml
CONC. OF S2       : 2.000 ug/ml
CONC. OF S3       : 3.000 ug/ml
CONC. OF S4       : 4.000 ug/ml
CONC. OF S5       : 5.000 ug/ml
CONC. OF S6       : 6.000 ug/ml
CONC. OF S7       : 7.000 ug/ml

CURVE FIT TYPE    : LINEAR POWER FIT WITHOUT TAILS
                  : Y = a+bX
                  : LINEAR AXIS FITTING
CURVE FIT Q.C.    : NO
SCALE GRAPH       : NO
SAMPLE            : T1 = 1:1          T2 = 1:1          T3 = 1:1
DILUTIONS
                  : T4 = 1:1
I.U. EQUATION     :
```

Curve Fit Data: Results Printout

DMPL Lab #JF Printed at 14:45 on 22.06.94 Page 9

TEST NO. : 02 W/L MODE : SINGLE DATE : 18.03.94
TEST NAME : MRX2 TEST FILTER : 410 nm TIME : 16:14
PLATE : PLATE2 REF. FILTER : * OPERATOR :

CALCULATION MODE : ENDPOINT
AVERAGE BLANKS:
A1 = 0.034 B1 = 0.034

AVERAGE BLANK =0.034

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	0.888	1.707	*****	*****	*****	*****	*****	*****	*****	*****	*****
B	BLANK	0.976	0.277	*****	*****	*****	*****	*****	*****	*****	*****	*****
C	-0.005	0.946	0.067	*****	*****	*****	*****	*****	*****	*****	*****	*****
D	-0.004	1.186	1.199	*****	*****	*****	*****	*****	*****	*****	*****	*****
E	0.056	1.626	1.159	*****	*****	*****	*****	*****	*****	*****	*****	*****
F	0.061	1.648	0.071	*****	*****	*****	*****	*****	*****	*****	*****	*****
G	0.306	2.103	0.886	*****	*****	*****	*****	*****	*****	*****	*****	*****
H	0.312	1.830	0.001	*****	*****	*****	*****	*****	*****	*****	*****	*****

CURVE FITTING

LINEAR POWER FIT WITHOUT TAILS
Y = +0.0587 -0.0224 X

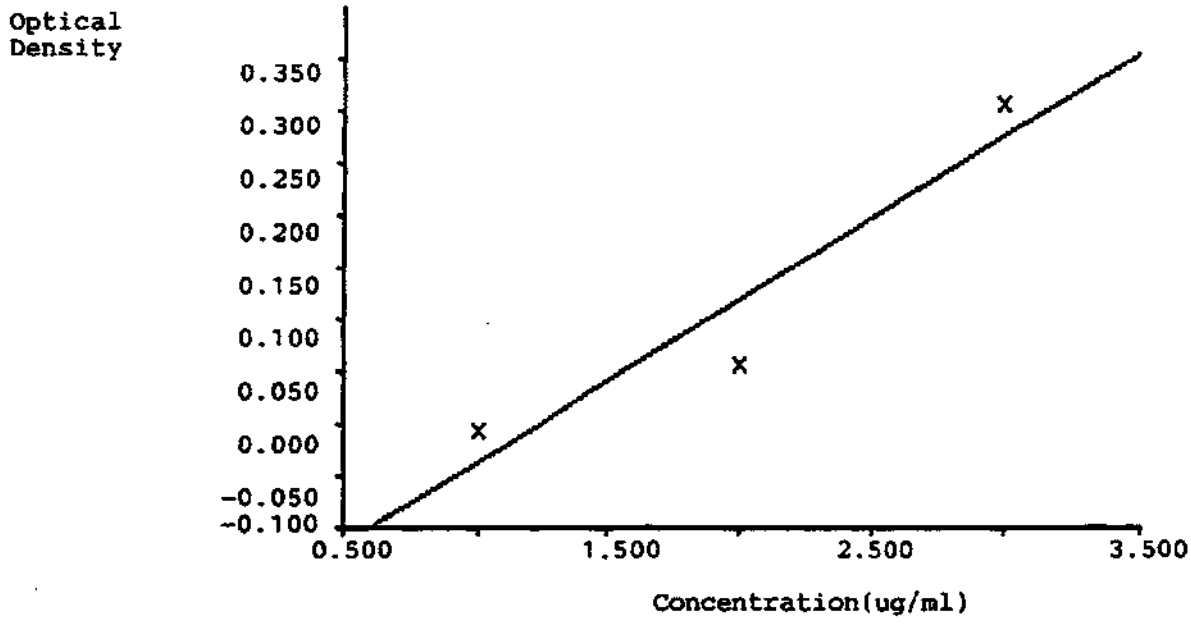
R-SQR = 0.5136

STANDARDS

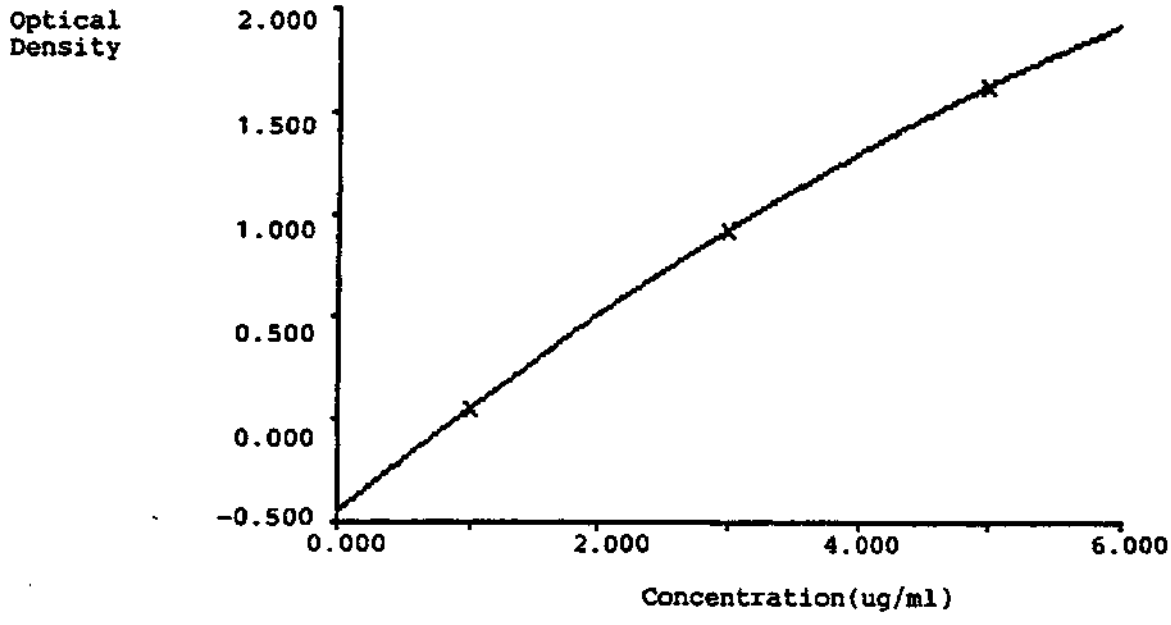
#	LABEL	LOC.	O.D.	MEAN	S.D.	C.V.	S.E.M.	CONC.	PRED.CONC.	%ERROR
S1	_____	C1	-0.005						1.001	0.053
		D1	-0.004	-0.005	0.001	-15.713	0.051	1.000		
S2	_____	E1	0.056						0.751	62.432
		F1	0.061	0.059	0.004	6.044	0.028	2.000		
S3	_____	G1	0.306						0.255	91.503
		H1	0.312	0.309	0.004	1.373	0.045	3.000		
S4	_____	A2	0.888						--------	********
		B2	0.976	0.932	0.062	6.677	0.083	4.000		
S5	_____	C2	0.946						--------	********
		D2	1.186	1.006	0.170	15.920	0.066	5.000		
S6	_____	E2	1.626						--------	********
		F2	1.648	1.637	0.016	0.950	0.034	6.000		
S7	_____	G2	2.103						--------	********
		H2	1.830	1.967	0.193	9.816	0.055	7.000		

Curve Fit Data

Linear Power Fit

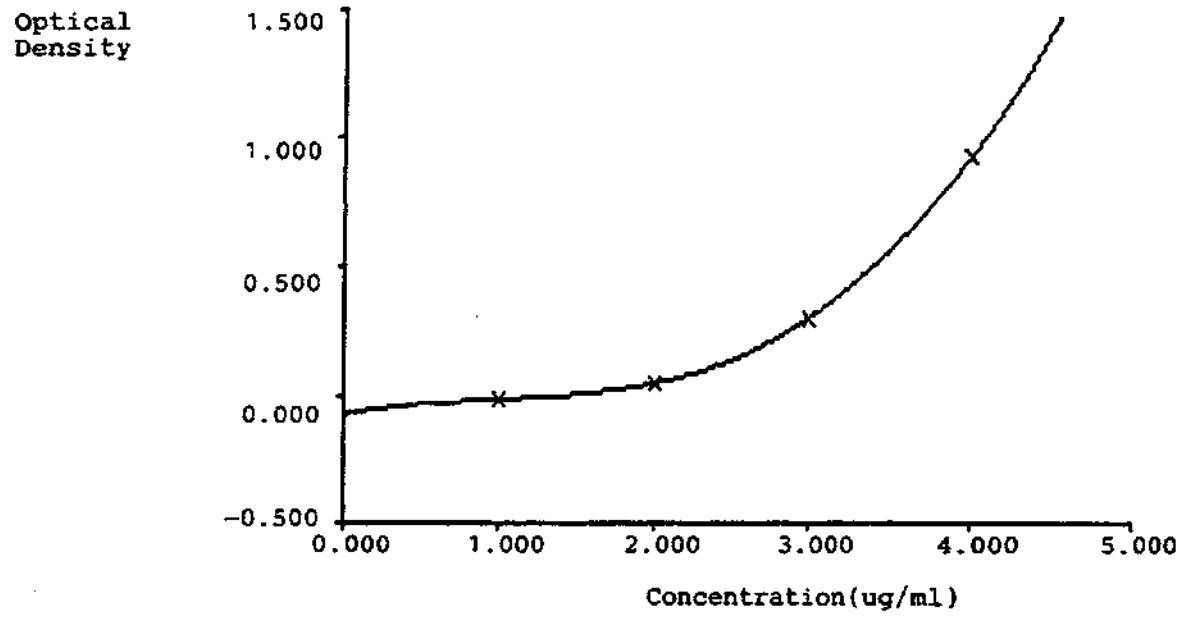


Quadratic Power Fit

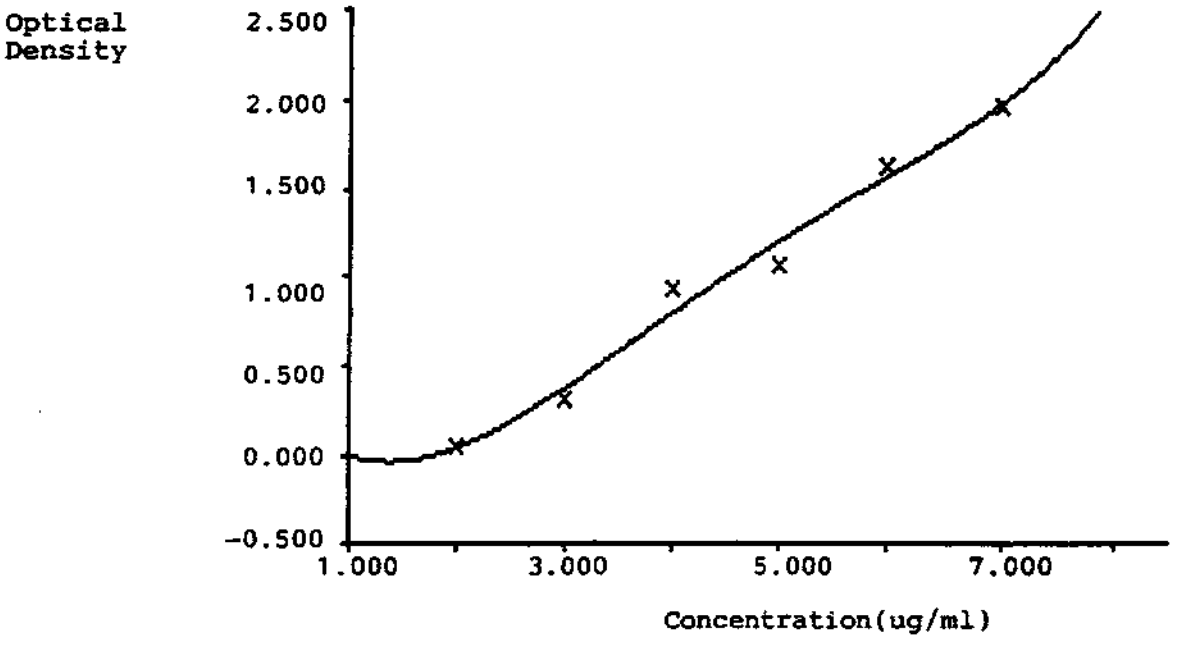


Curve Fit Data

Cubic Power Fit

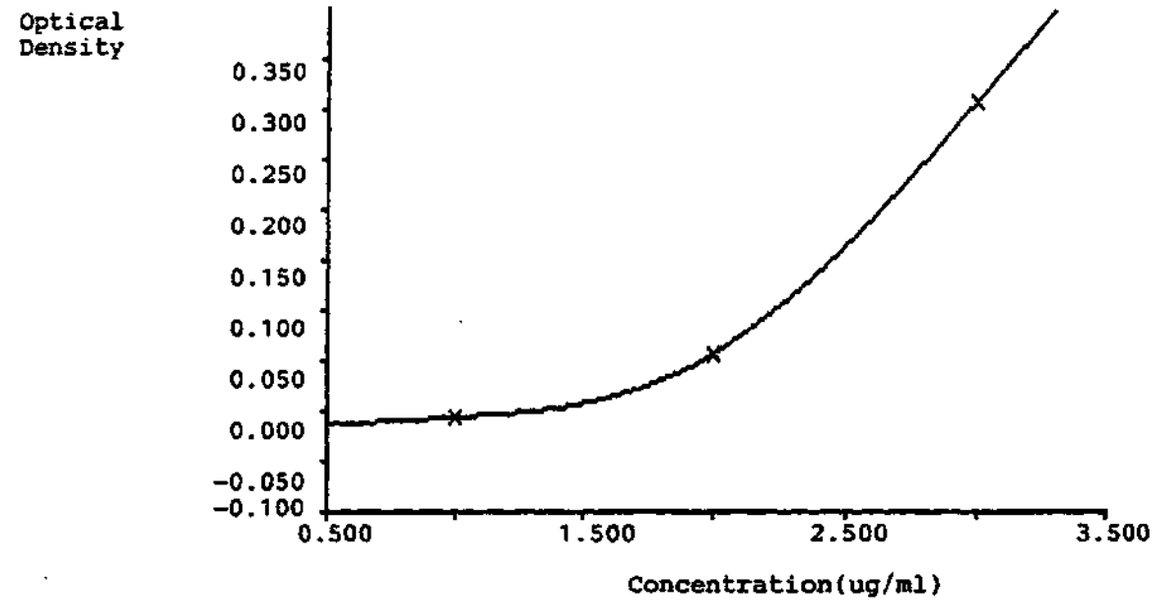


Quartic Power Fit

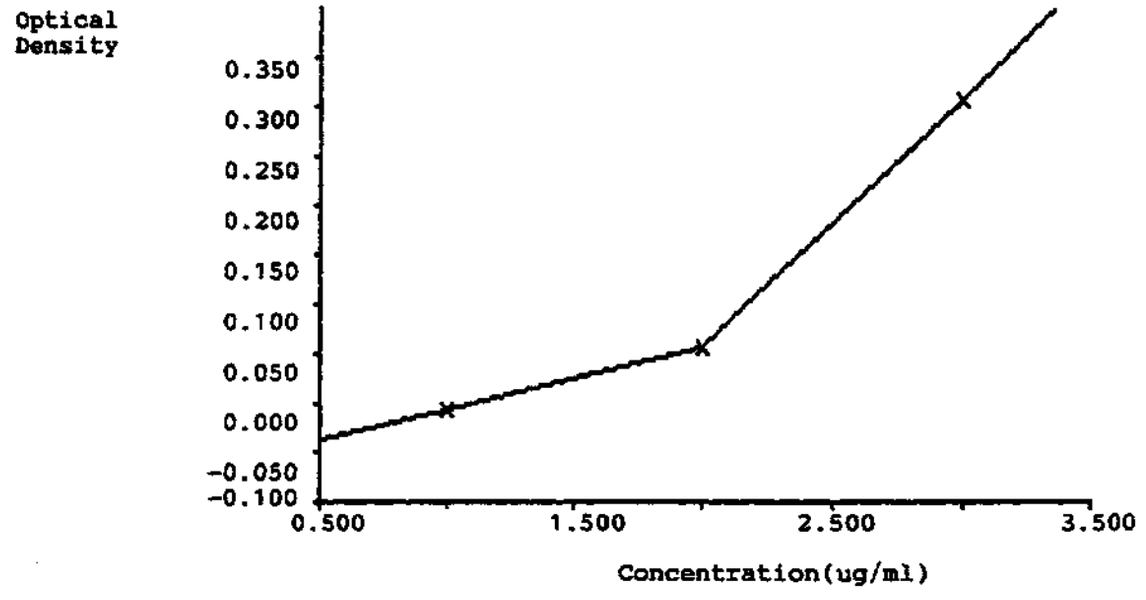


Curve Fit Data

Cubic Spline Fit

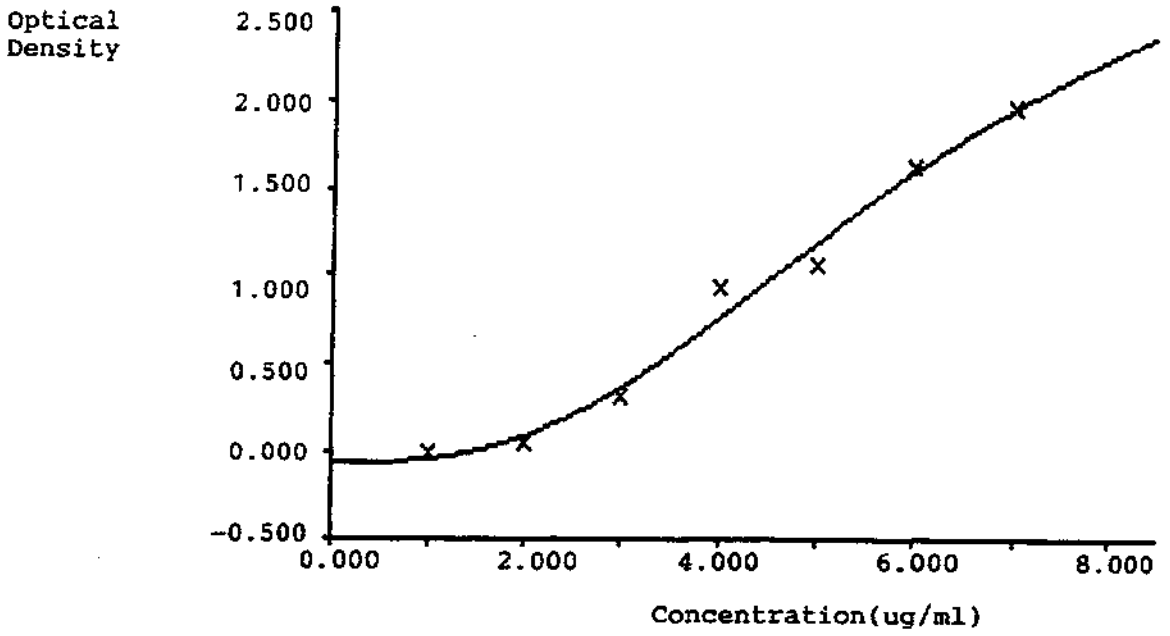


Polygon Fit

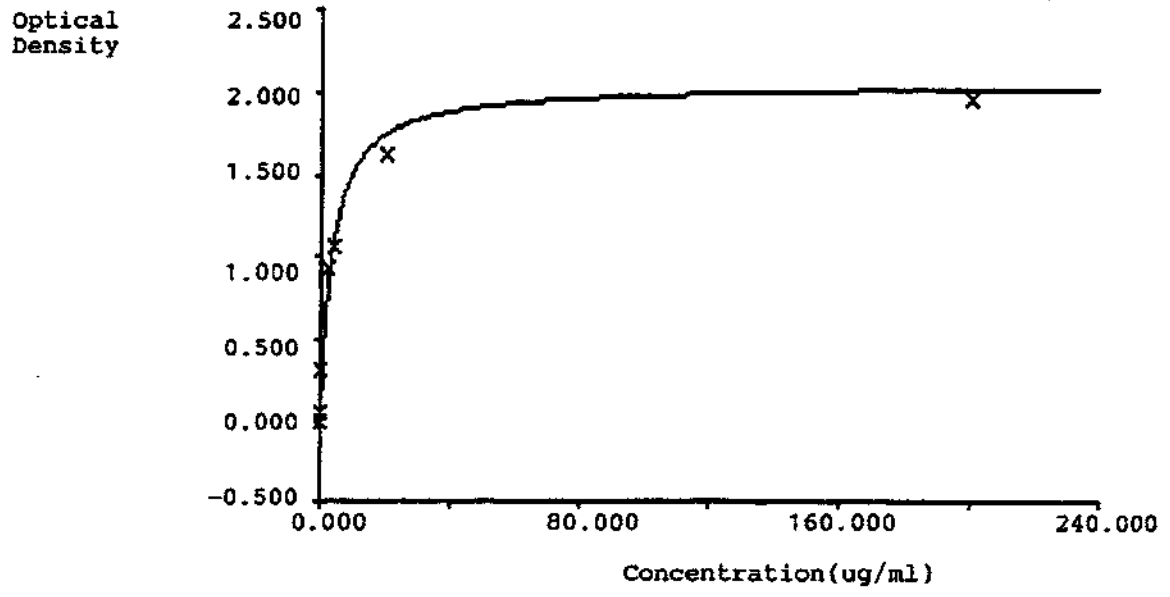


Curve Fit Data

Sigmoid Fit

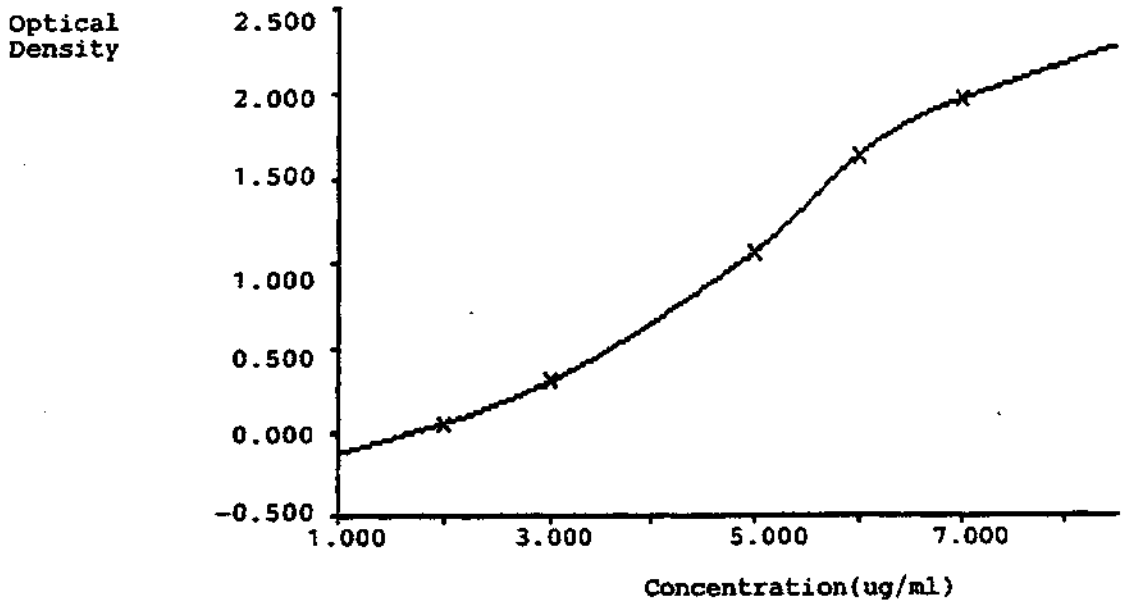


Michaelis-Menten Fit



Curve Fit Data

Akima Fit



Ratio Data: Test Printout

DMPL Lab #JF Printed at 14:45 on 22.06.94 Page 10

TEST NO: 03 TEST NAME: MRX3

PASSWORD :
WAVELENGTH MODE : DUAL
TEST FILTER : 410nm
REF. FILTER : 690nm
PLATE ID PROMPT : YES
O.D. MATRIX : YES
SHAKE : NO
BLANK MODE : AIR

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC1	T1	T9	T17	T25	T33	T41	T49	T57	T65	T73	T81
B	NC2	T2	T10	T18	T26	T34	T42	T50	T58	T66	T74	T82
C	NC3	T3	T11	T19	T27	T35	T43	T51	T59	T67	T75	T83
D	NC4	T4	T12	T20	T28	T36	T44	T52	T60	T68	T76	T84
E	CO1	T5	T13	T21	T29	T37	T45	T53	T61	T69	T77	T85
F	CO2	T6	T14	T22	T30	T38	T46	T54	T62	T70	T78	T86
G	PC1	T7	T15	T23	T31	T39	T47	T55	T63	T71	T79	T87
H	PC2	T8	T16	T24	T32	T40	T48	T56	T64	T72	T80	T88

Q.C. EQUATIONS :

AREA STATISTICS : NO

RATIO.

RATIO EQUATION : Sample/(PC*0.9)
I.U. EQUATION : Sample/15

Ratio Data: Results Printout

DMPL Lab #JF Printed at 14:45 on 22.06.94 Page 11

RATIO MATRIX

RATIO EQUATION : Sample/(PC*0.9)
: Sample/(PC*0.9)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.110	2.987	5.640	0.965	0.113	0.113	4.441	4.496	0.314	0.110	0.110	2.847
B	0.110	3.272	1.007	0.110	3.087	4.914	.0117	0.324	4.124	0.110	3.132	2.689
C	0.094	3.175	0.327	4.836	0.366	1.027	1.101	5.361	.0324	2.906	3.738	0.317
D	0.097	3.952	3.994	.0356	1.108	0.275	4.503	0.117	0.301	2.767	1.231	0.113
E	0.292	5.377	3.865	3.667	3.171	4.441	4.610	2.750	0.117	4.367	0.117	4.674
F	0.308	5.449	0.340	5.701	2.443	3.609	3.703	4.584	5.364	0.113	4.325	1.205
G	1.101	6.923	2.980	0.978	4.785	0.891	0.110	0.317	0.117	0.939	0.249	1.121
H	1.121	6.038	0.113	4.247	3.599	3.871	0.272	0.943	3.252	2.877	0.366	3.233

I.U. EQUATION : Sample/15
: Sample/15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.007	0.199	0.376	0.064	0.008	0.008	0.296	0.300	0.021	0.007	0.007	0.190
B	0.007	0.218	0.067	0.007	0.206	0.328	0.008	0.022	0.275	0.007	0.209	0.179
C	0.006	0.212	0.022	0.322	0.024	0.068	0.073	0.357	0.022	0.194	0.249	0.021
D	0.006	0.263	0.266	0.024	0.074	0.018	0.300	0.008	0.020	0.178	0.082	0.008
E	0.019	0.358	0.258	0.244	0.211	0.296	0.307	0.183	0.008	0.291	0.008	0.312
F	0.021	0.363	0.023	0.380	0.163	0.241	0.247	0.306	0.358	0.008	0.288	0.080
G	0.073	0.462	0.199	0.065	0.319	0.059	0.007	0.021	0.008	0.063	0.017	0.075
H	0.075	0.403	0.008	0.283	0.240	0.258	0.018	0.063	0.217	0.192	0.024	0.216

Difference Data: Test Printout

DMPL Lab #JF Printed at 14:45 on 22.06.94 Page 12

TEST NO: 04 TEST NAME: MRX4

PASSWORD :
WAVELENGTH MODE : DUAL
TEST FILTER : 410nm
REF. FILTER : 690nm
PLATE ID PROMPT : NO
O.D. MATRIX : YES
SHAKE : NO
BLANK MODE : AIR

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC1	T1	T9	T17	T25	T33	T41	T49	T57	T65	T73	T81
B	NC2	T2	T10	T18	T26	T34	T42	T50	T58	T66	T74	T82
C	NC3	T3	T11	T19	T27	T35	T43	T51	T59	T67	T75	T83
D	NC4	T4	T12	T20	T28	T36	T44	T52	T60	T68	T76	T84
E	CO1	T5	T13	T21	T29	T37	T45	T53	T61	T69	T77	T85
F	CO2	T6	T14	T22	T30	T38	T46	T54	T62	T70	T78	T86
G	PC1	T7	T15	T23	T31	T39	T47	T55	T63	T71	T79	T87
H	PC2	T8	T16	T24	T32	T40	T48	T56	T64	T72	T80	T88

Q.C. EQUATIONS :

AREA STATISTICS : NO

DIFFERENCE.

ALTERNATIVE FORMAT : NO
SUBTRACT : ADJACENT
SUBTRACT : COLUMNS
SUBTRACT. ORDER : 1st - 2nd

Difference Data: Results Printout

DMPL Lab #JF Printed at 14:45 on 22.06.94 Page 13

MRX

TEST NO.	: 04		W/L MODE	: SINGLE		DATE	: 18.03.94					
TEST NAME	: MRX4		TEST FILTER	: 410 nm		TIME	: 16:14					
PLATE	: PLATE4		REF. FILTER	: *		OPERATOR	:					

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.034	0.922	1.741	0.298	0.035	0.035	1.371	1.388	0.097	0.034	0.038	0.897
B	0.034	1.010	0.311	0.034	0.953	1.517	0.036	0.100	1.273	0.034	0.967	0.830
C	0.029	0.980	0.101	1.493	0.113	0.317	0.340	1.655	0.100	0.897	1.154	0.098
D	0.030	1.220	1.233	0.110	0.342	0.085	1.390	0.036	0.093	0.826	0.380	0.035
E	0.090	1.660	1.193	1.132	0.979	1.371	1.423	0.849	0.036	1.348	0.036	1.443
F	0.095	1.682	0.105	1.760	60.754	1.114	1.143	1.145	1.656	0.035	1.335	0.372
G	0.340	2.137	0.920	0.302	1.447	0.275	0.034	0.098	0.036	0.290	0.077	0.346
H	0.346	1.864	0.035	1.311	1.111	1.195	0.084	0.291	1.004	0.888	0.113	0.998

	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.888	#####	1.443	#####	0.000	#####	-0.017	#####	0.063	#####	-0.845	#####
B	-0.976	#####	0.277	#####	-0.564	#####	-0.064	#####	1.239	#####	0.137	#####
C	-0.951	#####	-1.392	#####	-0.204	#####	-1.315	#####	-0.797	#####	1.056	#####
D	-1.190	#####	1.123	#####	0.257	#####	1.354	#####	-0.733	#####	0.345	#####
E	-1.570	#####	0.061	#####	-0.392	#####	0.574	#####	-1.312	#####	-1.407	#####
F	-1.587	#####	-1.655	#####	-0.360	#####	-0.272	#####	1.621	#####	0.963	#####
G	-1.797	#####	0.618	#####	1.202	#####	-0.064	#####	-0.254	#####	-0.269	#####
H	-1.518	#####	-1.276	#####	-0.084	#####	-0.207	#####	0.116	#####	-0.885	#####

***** INDICATES VALUE OUT OF RANGE
INDICATES COMBINED DATA

Area Statistics: Test Printout

AREA STATISTICS : YES

	1	2	3	4	5	6	7	8	9	10	11	12
A				0	1	2	3					
B				0	1	2	3					
C				0	1	2	3					
D				0	1	2	3					
E				0	1	2	3					
F				0	1	2	3					
G				0	1	2	3					
H				0	1	2	3					

Area Statistics: Results Printout

	VALID WELLS	MEAN	S.D.	C.V.	S.E.M.
AREA 0	8	0.775	0.69	89.07 %	0.05
AREA 1	8	0.690	0.51	74.03 %	0.02
AREA 2	8	0.709	0.62	87.15 %	0.04
AREA 3	8	0.698	0.66	94.35 %	0.05
B	16	0.032	0.00	8.28 %	0.01
S	16	0.999	0.71	70.76 %	0.08
T	32	0.630	0.58	91.44 %	0.06
C	16	0.058	0.81	14.58	0.02
NC	4	0.817	0.77	94.66 %	0.03
PC	4	0.533	0.58	***** %	*****
CO	4	0.786	0.83	1.05	0.01
PR	4	0.951	0.96	1.00	0.05

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Appendix C: Curve Fitting

Linear Regression 144
Polygon Fitting 145
Non-Linear Curve Fitting 146
Quadratic, Cubic and Quartic Regression 147
Cubic Spline Curve Fitting 148
Other Equations 149
Michaelis- Menten 149
Sigmoid Equation 151
Akima Curve Fit 153
Axis Fitting 155
Semi-Log Fit 155
Log-Log Fit 155

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Appendix C provides theoretical notes for the curve fitting algorithms used in the Reader.

For all curve fitting routines, data is always plotted in the following way:

X axis Represents concentration

Y axis Represents measured variables (i.e., optical density, time, or rate of change with time)

This appendix describes the following types of curve fit:

Linear Regression	Straight line of best fit
Polygon Fitting	Joins successive data points with straight lines
Quadratic, Cubic, Quartic Regression	Curved line of best fit; similar to linear regression
Spline Curve Fitting	Curve fit which passes the curve through all the data points
Other Equations	Curve fits which include the Michaelis-Menten equation, the Sigmoid equation and the Akima curve fit

The following types of axis fit are also described:

Log-Log Fit

Semi-Log Fit

Linear Regression

Linear Regression is used to put a "best fit" straight line through a set of data points. The equation for a straight line is:

$$y = mx + c$$

where:

m = the slope
c = the y-intercept defining the line
x = the value for concentration
y = the value for the measured variable.

The linear regression formulae for determining the best straight line through a series of data points are:

$$m = \frac{N\sum x_i y_i - \sum x_i \sum y_i}{N\sum x_i^2 - \left(\sum x_i\right)^2}$$

$$c = \bar{y} - m \cdot \bar{x}$$

Where $\bar{x}$ and $\bar{y}$ are the mean values of x and y, i.e.:

$$\bar{x} = \frac{\sum x_i}{N}$$

The linear regression coefficient, **R**, is defined by:

$$R^2 = \frac{\left(N\sum x_i y_i - \sum x_i \sum y_i\right)^2}{\left(N\sum x_i^2 - \left(\sum x_i\right)^2\right)\left(N\sum y_i^2 - \left(\sum y_i\right)^2\right)}$$

When $\mathbf{R^2=1}$, all data points lie along the line of best fit. When $\mathbf{R^2=0}$, the data points are highly scattered.

Once a line of best fit has been established for a set of known Standards, the concentration of an unknown sample can be determined for a measured sample as follows:

$$\text{concentration} = \frac{y - c}{m}$$

Polygon Fitting

The polygon fitting procedure joins successive data points using straight lines.

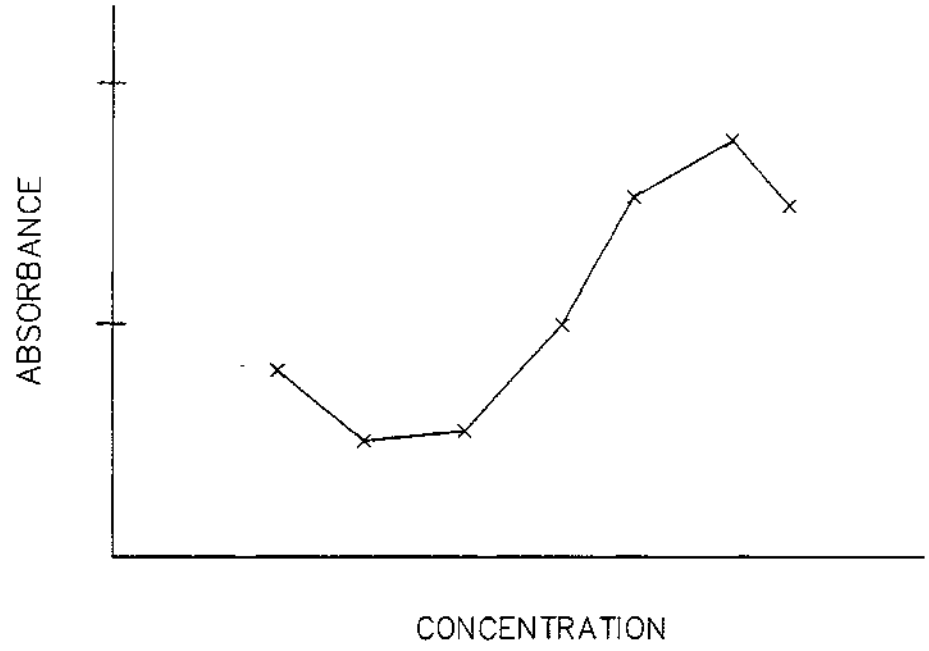

Figure C1 Polygon Fitting

Non-Linear Curve Fitting

The remaining fitting routines all produce curves rather than straight line plots.

The Reader will only calculate one concentration value for each measured variable. The user should examine the printed graph to determine if another curve would provide a better fit for the data points.

Some curves fit functions may not behave as expected if extrapolated beyond the range defined by the data points.

For example, in a particular test, the readings for a set of Standards increase in a non-linear fashion with increasing concentration. The user determines that a quadratic curve fit might give the best fit for such data. However, at concentrations lower than the concentration of the lowest Standard, the curve might turn sharply upwards (as shown in Figure C2).

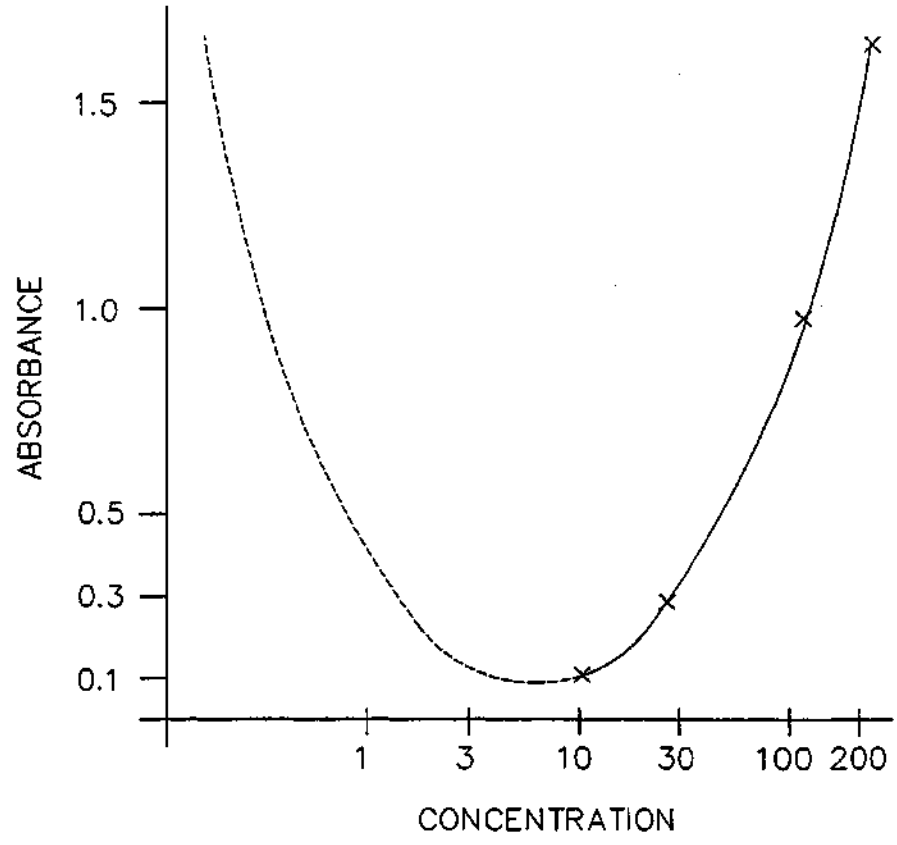

Figure C2 Using Tails

Quadratic, Cubic and Quartic Regression

Polynomial (quadratic, cubic and quartic) regression is best explained in parallel with linear regression, where the function to fit to experimental data can be written as:

$$y = a + bx^1$$

where:

b = the slope (corresponding to m in straight line equation)
a = the y-intercept of the line (corresponding to **c** in the equation for a straight line)

A quadratic equation is defined as:

$$y = a + bx + cx^2$$

A cubic equation as:

$$y = a + bx + cx^2 + dx^3$$

And a quartic equation as:

$$y = a + bx + cx^2 + dx^3 + ex^4$$

The procedures to fit such functions to experimental data are closely related to linear regression and have a similar mathematical derivation. Polynomial regression minimises the deviations of the data points from the polynomial equation (the square of the data point deviations are minimised since positive and negative deviations would otherwise cancel each other out).

There is an analogous regression coefficient **R**, which when $\mathbf{R^2=1}$ indicates that all the data points have been fitted to the curve; when $\mathbf{R^2=0}$, the data points are highly scattered.

Cubic Spline Curve Fitting

Cubic spline curve fitting passes a curve through all the data points. This is not a best fit algorithm since it assumes that the data set is perfect. The relevant equations are given below without explanation.

$$y = a_0 + a_1 x + \sum_{j=1}^{n-2} b_j \Phi_j(x)$$

Where:

$$\Phi_j(x) = \varnothing_j(x) - \left(\frac{x_n - x_j}{x_n - x_{n-1}}\right)\varnothing_{n-1}(x) + \left(\frac{x_{n-1} - x_j}{x_n - x_{n-1}}\right)\varnothing_n(x)$$

$$\Phi_j(x) = \begin{cases} 0 & x < x_j \\ (x - x_j)^3 & x \geq x_j \end{cases}$$

Difficulties can arise with spline fitting, if data is highly erratic or the curve bends sharply several times. The curve may break up at high concentrations (see Figure C3). The Reader will not be able to perform the calculations because of numerical rounding errors generated when attempting to fit the curve. Using a semi-log or log-log axis fit rather than a linear fit, will reduce this problem.

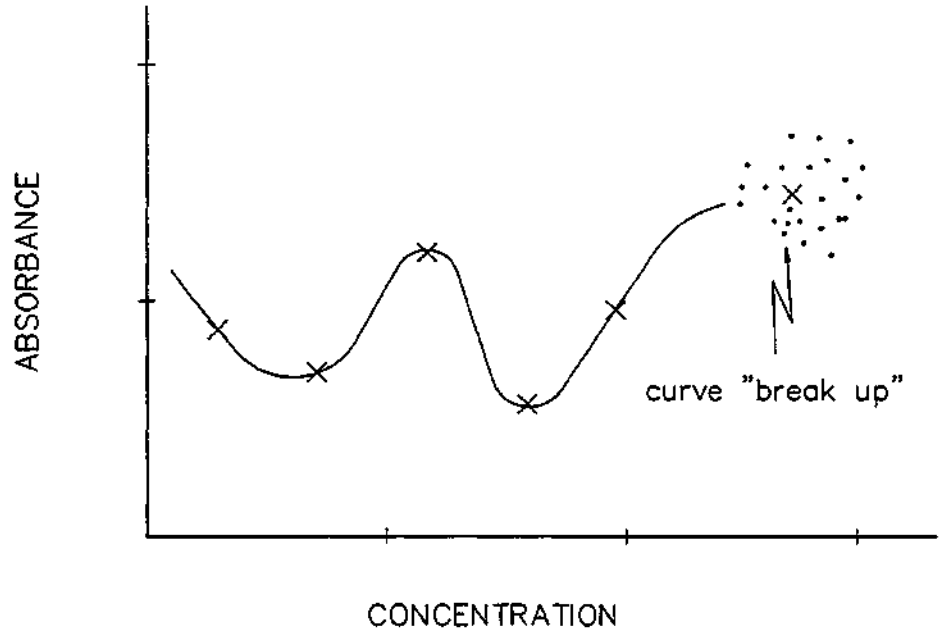

Figure C3 Spline Curve Break Up

Other Equations

The Reader uses three other types of equation to fit curves to data:

Michaelis-Menten	Used to describe enzyme kinetics, but its rectangular hyperbolic shape is useful for fitting curves to other types of experimental data
Sigmoid	A sigmoid, 'S' shaped curve is typical of many immunological reactions
Akima	Draws a 'natural looking' curve through all the data points

Michaelis-Menten

The Michaelis-Menten equation is used to describe enzyme kinetics, but its rectangular hyperbolic shape is useful for describing other experimental responses (see Figure C4).

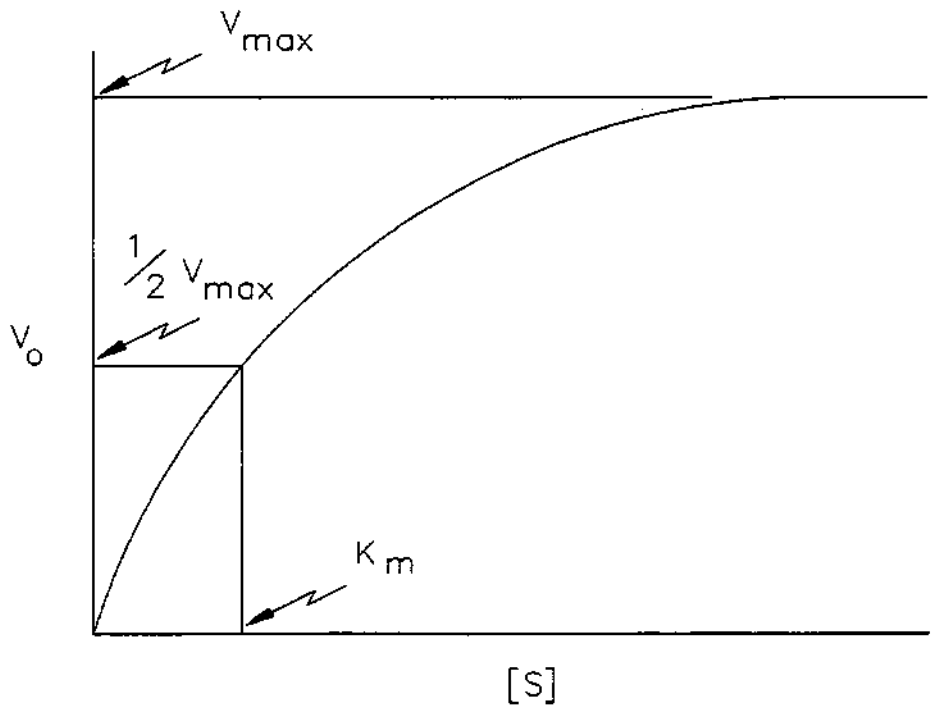

Figure C4 Michaelis-Menten Curve

The relationship between OD and concentration, **[x]**, can be written in the following general form:

$$OD = \frac{a[x]}{[x]+b}$$

Where **a** and **b** are constants.

It can be shown that **a** equals the limiting $\mathbf{OD_{max}}$ as **[x]**$\rightarrow\infty$, and that **b** is the concentration at half $\mathbf{OD_{max}}$. The equivalent formula applied to enzyme reaction rates is:

$$V_0 = \frac{V_{max}[S]}{[S]+K_m}$$

Where:

[S] =	Enzyme concentration
$\mathbf{V_{max}}$ =	Maximum reaction velocity
constant $\mathbf{K_m}$ =	Enzyme concentration at which the reaction velocity is half $\mathbf{V_{max}}$.

The constant $\mathbf{K_m}$ is known as the Michaelis constant.

For practical applications it is best to transform the Michaelis-Menten equation into a linear form, known as the Lineweaver-Burk equation.

$$\frac{1}{OD} = \frac{1}{OD_{max}} + \frac{K_m}{OD_{max}} \cdot \frac{1}{[x]}$$

If **1/OD** is plotted against **1/[x]**, a straight line is obtained whose slope is $\mathbf{K_m/OD_{max}}$, with an intercept on the **1/OD** axis $\mathbf{1/OD_{max}}$ and an intercept on the **1/[x]** axis of $\mathbf{1/K_m}$. The Reader performs this calculation automatically and determines the best straight line fit by linear regression. $\mathbf{OD_{max}}$ (printed as $\mathbf{V_{max}}$) and $\mathbf{K_m}$ are evaluated and printed out.

Sigmoid Equation

Many immunological reactions are characterised by a S-shaped or sigmoid curve.

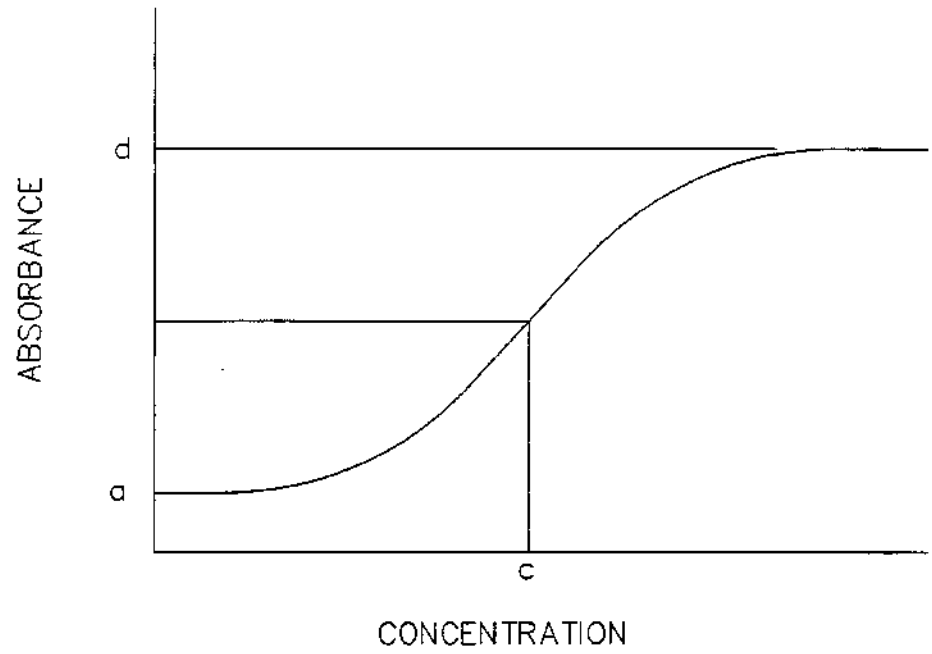

Figure C5 Sigmoid Curve

The Sigmoid equation can be written as:

$$Y = \frac{[a-d]}{\left[1+\left(\frac{X}{c}\right)^{b}\right]} + d$$

Note: This equation is also known as Rodbard's Four Parameter equation; the four parameters are:

- **a** minimum response
- **b** shape factor (determines the gradient of the curve)
- **c** the response midway between the maximum **(d)** and the minimum **(a)** responses
- **d** maximum response

*Note: This definition assumes a positive value for **b**. Where **b** is negative, the definitions for parameters **a** and **d** are reversed.*

The Reader contains an efficient algorithm for estimating the four parameters and determining the best fit for the sigmoid curve. The curve requires at least four Standards (data points): one for each of the four parameters. It is recommended that at least eight Standards are defined to ensure satisfactory statistical significance. The Reader will allow a maximum of 24 Standards.

The sigmoid curve algorithm is iterative, requiring many complex calculations and the Reader may take some time to process the results. If the Reader has difficulty fitting the data to the curve, processing will continue, either until the Reader has processed the data, or until you select **ABORT**. If you have aborted the data processing, examine the data carefully, to see if the number of Standards could be altered to better define the curve.

Akima Curve Fit

The Akima option constructs a smooth curve through the data point. The fitted curve will appear smooth and natural and approximates to a manually drawn curve.

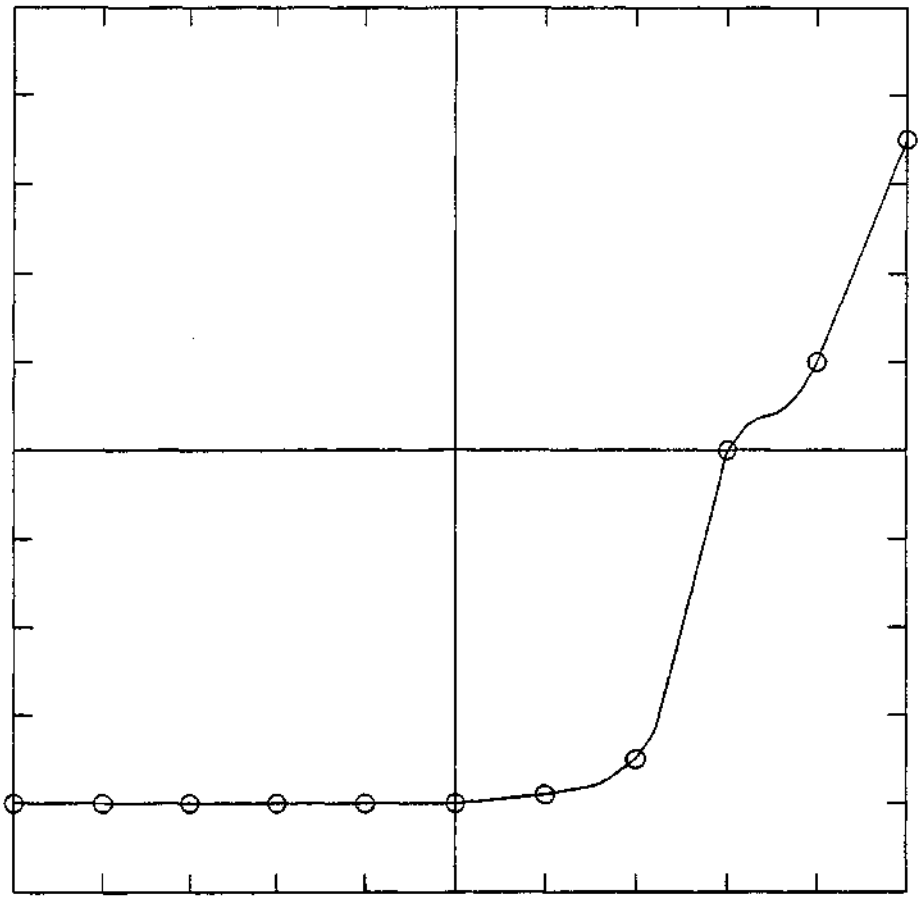

Figure C6 Points joined by the Akima Curve Fit Method

The Akima curve fit consists of a set of polynomials which are applied to the set of data points. The slope of the curve at each point is determined by these polynomials. The Akima fit is based on the assumption that each data point is determined locally by five points: The point of interest and two points on either side of it.

Note: The Akima method is discussed more fully in the Journal of the Association of Computing Machinery, Vol 17, No. 4 (October 1970, pages 589-602) "A new Method of Interpolation and Smooth Curve Fitting Based on Local Procedures.

The slope of the curve

Numbering the points 1, 2, 3, 4, 5, the slope of the curve at the point of interest (point 3) is assumed to be determined by:

$$t = \frac{(|m_4 - m_3|m_2 + |m_2 - m_1|m_3)}{(|m_4 - m_3| + |m_2 - m_1|)}$$

Where t is the slope of the curve, and m1, m2 m3, and m,4 are the slopes of the following line segments:

$$\overline{12}, \overline{23}, \overline{34}, \overline{45}$$

Interpolating between a pair of points

With the slopes of the points determined, the portion of the curve between two points is calculated so that the curve:

passes through both points

the slopes at the data points are the same as previously determined.

The portion of the curve is expressed as a uniquely determined polynomial in the form:

$$y = p_0 + p_1(x - x_1) + p_2(x - x_2)^2 + p_3(x - x_1)^3$$

where

$$p_0 = y_1$$

$$p_1 = t_1$$

$$p_2 = \frac{3(y_2 - y_1)/(x_2 - x_1) - 2t_1 - t_2}{x_2 - x_1}$$

$$p_3 = \frac{t_1 + t_2 - 2(y_2 - y_1)/(x_2 - x_1)}{x_2 - x_1}$$

Axis Fitting

The normal method of fitting data to the axes of a graph is to use linear scales for the x (concentration) and y (measured variable) axes. Two other axis fitting methods are available:

Semi-Log Fit

Log-Log Fit

Semi-Log Fit

All x are transformed in the following way:

$$x = \log(\text{concentration})$$

Thus, to obtain the concentration of a sample using linear regression:

$$\text{concentration} = \text{antilog}\left(\frac{y-c}{m}\right)$$

Log-Log Fit

All x and y values are transformed as follows:

$$x = \log(\text{concentration})$$

$$y = \log(\text{measured variable})$$

Thus to obtain the concentration of a sample using linear regression:

$$\text{concentration} = \text{antilog}\left(\frac{y-c}{m}\right)$$

Note: These examples use linear regression, but Log-Log and Semi-Log Axes Fits may be used with other curve fitting routines as well.

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Index

—A—

Air blanking mode, 33
Akima curve fit, 4, 58, 153
Area statistics, 36–38
  editing, 79
Arithmetic operators in equations, 38, 43, 46, 49, 52, 55
Automatic template definition, 29–30
Average blanking mode, 34
Axis fit for curve fit graphs, 56, 60
axis fitting, 155

—B—

B/S/T/C Wells, 24
Backup, 90
Barcode, 22
Barcode scan, 78
Batch option, 92
Beer-Lambert law, 10
Blank wells, 33, 77, 92
Blanking, 3, 10, 33–35
  air blanking mode, 33
  average blanking mode, 34
  column blanking mode, 35
  individual blanking mode, 34
  last blanking mode, 34
  paired blanking mode, 35
  plate blanking mode, 34
  row blanking mode, 35
  whole blanking mode, 35
Brackets in equations, 38, 39, 40, 43, 46, 48, 49, 52, 53, 54, 55

—C—

C.V.. *See* Coefficient of variation
Calculation mode, 32
Clock function, 3
Coefficient of variation, 41, 43, 48, 49, 91, 92
Column blanking mode, 35
Computer control commands
  blank modification, 107–8
  computer control, 110
  display control, 110
  filter select, 106
  print control, 108–9
  read mode, 106
  reduced matrix, 110
  start/stop, 104
  step x/y, 103
Constant value calculation mode, 8
cubic spline curve fitting, 148
curve fitting algorithms, 143
Curve fitting output option, 56–64
  axis fit, 60
  curve fit output, 79–83
  curve fit quality control limits, 61–62
  curve fit type, 58–60
  editing standards, 80–82, 80–82
  international units equation, 63
  printing as a graph, 80
  reagent concentration, 57–58
  results format, 57
  results table, 64
  sample dilution, 62–63
  scale graph, 62
  slope and Y axis intercept, 60
  stored curve, 57
  tails, 61
  units, 61

—D—

Data control
  response with data, 111–12
  responses without data, 113
  uncontrolled transmission, 113
Data storage, 4
Define template prompt, 24
Difference matrix output option, 64–66
  adjacent, 65
  half, 65
  subtraction order, 65–66
Directories
  printing, 69

—E—

Editing a test, 66–68
  editing test options, 67
  editing the output options, 68
  editing the template, 67
  making a copy of, 66
Endpoint Program. *See* also the Reader User Manual
  features and specifications, 3
  menu, 19
  menu language, 3
  program load time, 4
  results output, 4
  version number, 90
  Wavelength modes, 4

—I—

Individual blanking mode, 34
Installation
  of Endpoint in a PR 1100, 11
  re-installing Endpoint in a PR 2100, 14
  Uninstall option, 91
inverse assay, 47, 50, 52

—L—

Last blanking mode, 34
linear regression, 144
Linear regression calculation mode, 8

—M—

Menu language, 3
Menus, 89
  Menus option, 89
  short menus, 89
Michaelis-Menten curve fit, 4, 58, 81
Michaelis-Menten equation, 149–50
Microplate Reader, 3. *See* also the Reader User Manual
  checksum algorithm, 102
  clock function, 3
  computer control commands, 102–11
  curve fitting algorithms, 143
  data format, 111–13
  data storage, 4
  handshaking, 100–101
  hardware specification, 97
  linking with an Apple Macintosh, 99
  linking with an IBM-compatible PC, 98–99
  main menu, 19
  plate identification, 3
  short cut test selection, 3
  software specification, 100
  tests per plate, 3
Multiple wavelength mode, 8

—N—

non-linear curve fitting, 146
Number of samples, 75–77

—O—

Optical path, 9–10
Output options
  editing the, 68
  inserting into a test, 44–66, 44–66

—P—

Paired blanking mode, 35
Password
  adding to a test, 45
Pathlength, 10
Photometry, theory of, 7
Plate blanking, 34
Plate ID prompt, 22, 77
Plate identification, 3
polygon fitting, 145
polynomial regression, 147
Printing
  directories, 69
  tests, 68–69

—Q—

QC equation components, 43–44
quadratic, cubic, and quartic
  regression, 147
Quality control equations, 38–44

—R—

Ratio matrix output option, 52–55
  ratio and IU equation components,
    55
Reading a plate, 89
Recalling plates, 83–84
Restore option, 91
Results matrix, 36
Row blanking mode, 35
Running a test, 73–83
  number of samples, 75–77
  repeating, 83
  test number, 74–75

—S—

Shake
  inserting in a test, 22–23
  shake modes, 23
  shake time, 22
Short cut test selection, 3
Short menus, 89
Sigmoid equation, 151
Single and dual wavelength
  modes, 7–8
Spectrum option, 92
Stats option, 91, 92

—T—

Template
  define template prompt, 24
  defining automatically, 29–30, 29–
    30
  defining manually, 25–28
  editing, 67
Test
  creating a new, 20–66
  editing a, 66–68
  editing test options, 67
  making a copy of, 66
  password protected, 21
  repeating, 83
  running a, 73–83
Test name, 21
Test number, 74–75
Test Number, 20–21
Tests
  printing, 68–69
Tests per plate, 3
Threshold matrix output option,
  45–52
  threshold limits, 51–52

—U—

Upgrading
  assay protocol files in a PR 2100, 15–16
  from a previous version of Endpoint, 14–15
User Wells, 27
Utility menu
  accessing the, 28
  manual mode, 88–89
Utility Menu, 87–93

—V—

Variables, 79
Version number of Endpoint Program, 90

—W—

Wavelength modes, 4, 30–32
Wavelength Modes, 7–8
Well location calculation mode, 8
Well types
  blank, standard, test, or control, 24
  creating new, 27
  orientation, 25
  replicates, 25
Whole blanking mode, 35